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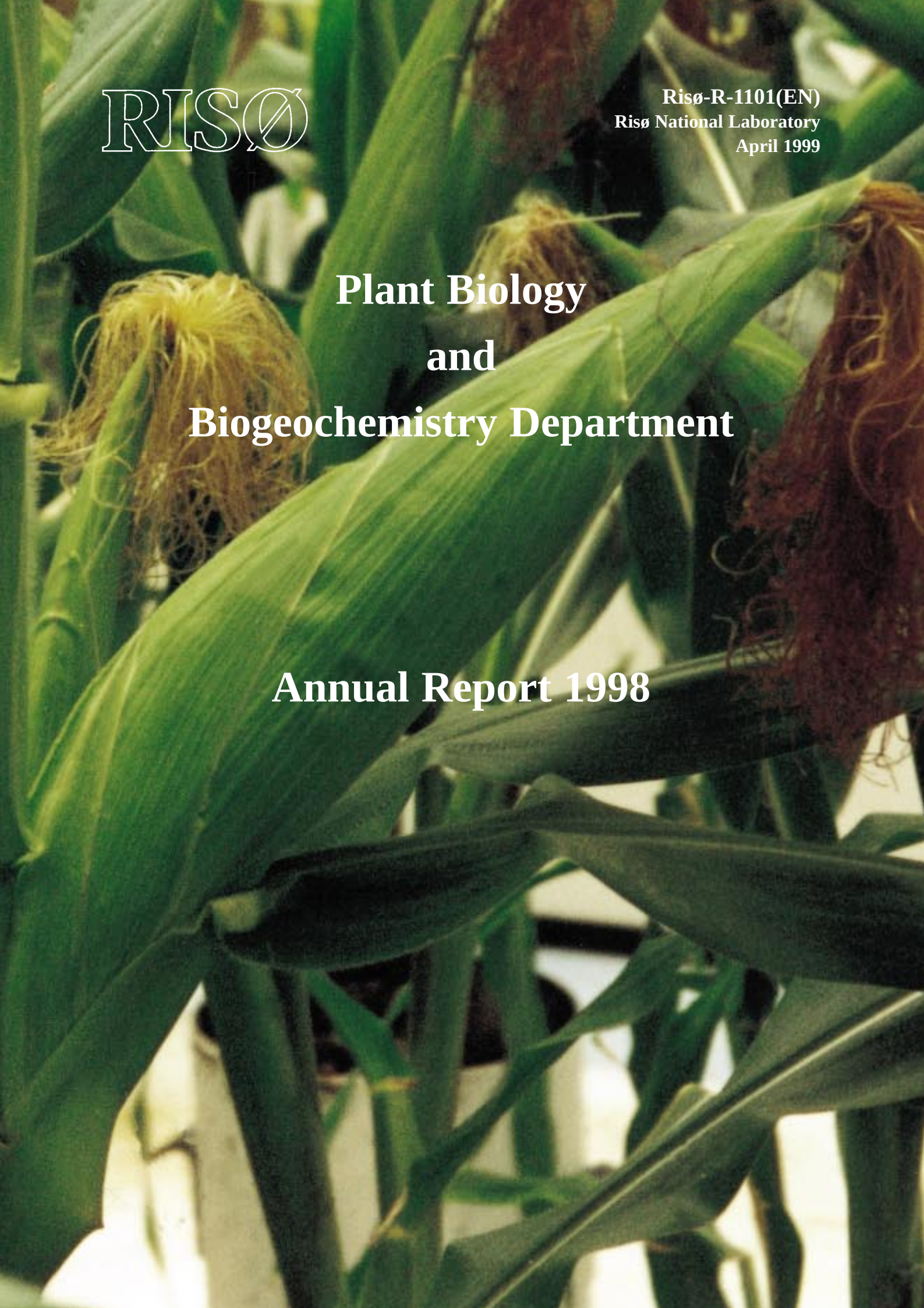

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April 1999

Plant Biology and Biogeochemistry Department

Annual Report 1998

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Plant Biology
and
Biogeochemistry Department

Annual Report 1998

**Edited by A. Jensen, G. Gissel Nielsen, H. Giese,
K.K. Nielsen, L. Rasmussen, S. Rasmussen, H. Østergård**

**Risø National Laboratory, Roskilde, Denmark
April 1999**

Abstract

The annual report from the Plant Biology and Biogeochemistry Department aims to provide a summary of our research and achievements and to give an idea of the research directions in the Department.

The Department is engaged in research to establish the scientific basis for new methods in industrial and agricultural production. Through basic and applied experimental research, the Department aspires to develop methods and technology for industrial and agricultural production, exerting less stress and strain on the environment. The research approach in the Department is mainly experimental. In the autumn of 1997 it was decided to reorganize and expand the Department and in 1998 the Department includes six research programmes and special facilities.

Selected departmental research activities during 1998 are introduced and reviewed in seven chapters: 1. Introduction, 2. Plant-Microbe Symbioses, 3. Plant Products and Recycling of Biomass, 4. DLF-Risø Biotechnology, 5. Plant Genetics and Epidemiology, 6. Biogeochemistry, 7. Plant Ecosystems and Nutrient Cycling.

The Department's contribution to education and training are presented. Lists of publications, papers accepted for publications, guest lectures, exchange of scientists, lectures and poster presentations at international meetings are included in the report. Names of the scientific and technical staff members, visiting scientists, Postdoctoral fellows, Ph.D. students, M.Sc. students and apprentices are also listed.

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1 Introduction

1.1 The Plant Biology and Biogeochemistry Department

Research Objectives
The Department is engaged in research to establish the scientific basis for new methods in industrial and agricultural production. Through basic and applied experimental research, the Department aspires to develop methods and technology for industrial and agricultural production, exerting less stress and strain on the environment.

Approach
The Department's expertise spans a wide range of subjects including atmospheric chemistry, chemical kinetics in the liquid and gas phase, geochemistry, geochemical modelling, hydrochemistry, analytical chemistry, process chemistry, plant molecular biology, plant pathogenicity, plant genetics, population biology, plant nutrition, nutrient cycling, ecophysiology, terrestrial ecology and ecology of trace elements.

The results of research and development are disseminated internationally to companies, institutions, organizations and public authorities through scientific publications, research reports, lectures and posters at scientific - and other professional meetings, personal communication with collaborators and through teaching courses at universities.

The research and development activities in the Department are planned for three years and reassessed every year. The research activities are mainly funded directly from the government or from National Science Research Councils. However, national and European research programmes, private foundations and commercial contracts also make substantial contributions to the total budget of the Department.

1.2 Research Programmes

1.2.1 Plant-Microbe Symbioses
In 1998 the programme received a large grant for 5 years from The National Science Foundation to establish a research centre for Plant-Microbe Symbioses. The programme has been established in new buildings and scientists with expertise in the required scientific fields have been recruited. The new facilities and instrumentation are in operation.

The Plant-Microbe Symbioses Programme aims to characterize genes and processes involved in the molecular interaction and compound exchange mechanisms operating during the establishment and maintenance of a symbiotic relationship between plants and micro-organisms. The symbioses under study are: *Rhizobium*/pea, mycorrhiza/pea and barley and *Erysiphe graminis* f.sp. *hordei*/barley. A feature common to all plant-microbe symbioses is the integration of the micro-organism into the interior of a viable plant cell. In all plant-microbe symbioses a symbiotic interface emerges by formation of a tissue specific plant-derived membrane. Molecular communication between the symbionts must proceed via this membrane and the interface thus constitutes the major point of control of the symbiotic relationship.

1.2.2 Plant Products and Products Quality
The programme was established in 1998 with the purpose of developing plant lines of higher quality by use of modern molecular technologies. This included a production of transgenic plants, cereals in particular, in order to create an overproduction of enzymes or to alter specifically the gene expression to modify the nutritional quality (phosphorus and nitrogen) or modulate the polymer content like lignin.

The growing public interest in the fact that our lives depend upon the availability of ever-diminishing reserves of petroleum has given momentum to the search for renewable resources as chemical raw materials. The carbohydrates potentially available from biomass waste products, such as wood chips and agricultural residues or purposely grown crops, represent one conceivable alternative. The programme contributes to the scientific basis for developing the wet oxidation technique for pre-treatment and conditioning of biomass, straw and wood chips. Wet oxidation readily solubilizes lignin in straw and the product is susceptible to enzymatic treatment and fermentation. In the field of upgrading biomass, the programme aims at converting straw to ethanol by means of wet oxidation followed by fermentation.

1.2.3 DLF-Risø Biotechnology
The programme was initiated in 1998 through the establishment of the biotechnology consortium among DLF-TRIFOLIUM A/S, Research Division, and Risø National Laboratory. The programme is co-funded by The Danish Research Councils, THOR programme. The programme aims to characterise and control key genes involved in the regulation of flowering in ryegrass (*Lolium perenne* L.). The overall objective is to develop transgenic high value grass plants, which are incapable of producing stems and flowers during grassland farming (*biological encapsulation*) and with highly improved quality for agronomic use.

Forage grass is by far the largest agricultural crop in the EU, occupying a total of 20 mill. ha in frequent rotation. Compared to other agricultural crops, the grass field has a very positive environmental profile due to limited use of pesticides, limited seepage of nutrients and the allowance of a large diversity of wild plant species, insects and animals. However, the value of grass as cattle fodder is limited by the fact that especially the grass stems contain high amounts of low digestible, mainly lignin-containing, compounds.

The programme employs 15 newly engaged persons and started in autumn 1998, when the group moved into new laboratory facilities at Risø National Laboratory.

1.2.4 Plant Genetics and Epidemiology

The programme intends to establish the scientific basis for breeding crop plants with new and stronger resistance to diseases and with improved nutrient efficiency. Crop plants, highly resistant to diseases and efficient in nutrient uptake, are a prerequisite for low input plant production. The research includes modelling of evolutionary processes and the detection of gene products, which are important for resistance and virulence. The host-pathogen system, barley and barley powdery mildew *Erysiphe graminis* f.sp. *hordei*, is used as a main model system for an obligate fungus.

Much of the research effort is devoted to the identification of genetic markers for rapid and effective identification of genotypes. Studies of plant population biology are necessary to predict possible consequences of using new genotypes possessing transgenes. Introgression of genes from crop plants to their wild relatives is being studied to assess the risks of using genetically modified plants in the plant production. Oilseed rape and wild *Brassica* species are used as model systems for these studies.

1.2.5 Biogeochemistry

The research programme aims at understanding fundamental chemical processes and developing methods for chemical analysis of agricultural products, organic micro pollutants in the food chain.

The research focuses on the occurrence, transport, turnover and effects of trace elements and organic micro contaminants in agricultural and forest ecosystems. Trace elements are followed from the soil or the atmosphere to the crop, and through the human food chain. The effects of air pollution and global change are studied, both at the plant and at the ecosystem level. Major emphasis is placed on the development of new methods and processes, which can form the basis of an environmentally benign and sustainable plant production.

1.2.6 Plant Ecosystems and Nutrient Cycling

The research emphasizes biological, physiological, biochemical and chemical processes involved in the transfer of plant nutrients through the Soil-Plant-Air-Continuum. A better understanding of the biological and chemical processes in the soil-plant-atmosphere system will lead to a reduced requirement for energy-consuming fertilizers and to a reduced loss of nutrients. The microbial biomass in the soil is of fundamental importance to the mineralization of nutrients in plant residue and animal manure. Special attention is directed towards pro cesses involved in the cycling of nitrogen and turnover of organic matter. Nitrogen is essential for plants, but also a risk to the environment through the leaching of nitrate and volatilization of some other nitrogen compounds. Information about these processes will provide the basic knowledge needed for a sustainable plant production.

In order to fertilize the different parts of the fields in accordance with the natural variation in topography, texture and soil fertility, we are developing systems to continuously measure plant vigour in relation to the position of the machinery in the field.

1.3 Research Projects

Detailed information about all research projects in the Department can be obtained from **WWW** at **http://www.risoe.dk/pbk**.

1.4 Organization of the Plant Biology and Biogeochemistry Department

During 1997 it was decided to reorganize and expand the Department and in 1998 the Department includes the following six research programmes and special facilities:

Research Programmes

Plant Microbe-Symbioses
Head: Henriette Giese.
.
Plant Products and Products Quality
Head: Søren K. Rasmussen (constituted).

DLF-Risø Biotechnology
Head: Klaus K. Nielsen.

Plant Genetics and Epidemiology
(*Including the experimental farm, Dyskærgård and field experiments)*
Head: Hanne Østergård.

Biogeochemistry
Head: Lennart Rasmussen (constituted).

Plant Ecosystems and Nutrient Cycling
(Including growth chambers, Risø Environmental Risk Assessment Facility (RERAF), green houses)
Head: Gunnar Gissel Nielsen.

Special Facilities

Risø High Dose Reference Laboratory
Head: Arne Miller.

Risø Integrated Environmental Facility (RIMI)
Head: Kim Pilegaard.

Head of Department

Arne Jensen

2 Plant-Microbe Symbioses

The Centre for Plant Microbe Symbioses has been established in new buildings; we have recruited new scientists with expertise in the required scientific fields and the instruments and facilities are in operation. The focus of the programme is to characterize genes and compounds, important for functional symbioses, exemplified by *Rhizobium* and pea, different mycorrhiza plant associations and *Erysiphe graminis* and barley. Physiological and molecular approaches are employed to identify the genes and processes.

2.1 *Rhizobium*/Pea Symbiosis

2.1.1 Characterization of an Amino Acid Carrier in the Symbiosome Membrane

(L. Rosendahl, A. Rudbeck)

The membrane interface between the partners in plant-microbe symbioses constitutes a regulatory site for the function of the symbiosis. The technology we have developed to study transport processes in the membranes of the interface has been used to identify and characterize a carrier in the symbiosome membrane which directs transport of amino acids from the microbe to the plant in legume root nodules. The carrier is likely to be involved in a carbon-nitrogen exchange mechanism whereby the energy generating system of the bacteroid may be regulated.

2.1.2 Development of Methods to Determine if Symbiosomes Directly Import Aspartate Minotransferase

(G. Saalbach, L. Rosendahl)

Symbiosomes consisting of bacteroids, including membrane interface have been isolated and experiments have been carried out to confirm indications that aspartate aminotransferase (AAT2) can be directly imported into isolated symbiosomes. *In vitro* transcription and translation as well as a protease assay have been established. In addition, the generation of vector constructs, containing AAT2 linked to the GFP reporter gene, EGFP, has been initiated. These will be used to study the expression of AAT2 in bacteria and in transgenic plants. In the latter, the intracellular localization of AAT2-EGFP will be studied.

2.1.3 Establishment of 2-D Gel Electrophoresis to Carry out Proteom Analyses of Symbiosome Fractions

(G. Saalbach, L. Rosendahl)

Two-dimensional (2-D) protein electrophoresis has been established with the aim of performing proteome analysis on symbiosomes. The Pharmacia MultiphorII System has been used with precast strips for isoelectric focussing and SDS-gels. Conditions have been optimized to separate symbiosome protein preparations in pH 3-10 and pH 4-7 gradients. Symbiosomes were purified from root nodules of peas. They were subfractionated to obtain the symbiosome membrane fractions (SM) and the peribacteroid space (PBS). For comparisons, symbiosomes were isolated from 3 and 6 weeks old nodules. SM fractions were washed with carbonate to dissociate attached membrane proteins. 2-D gels of the PBS and SM fractions are currently analysed, using the PDQuest image analysis software (Bio-Rad).

2.2 Mycorrhiza/Plant Symbioses

2.2.1 Study of Inter Fungal Differences in Hyphal P Transport

(I. Jakobsen, S. Smith, A. Smith)

The P transport by mycorrhizal fungi has been investigated in collaboration with visiting scientists from the University of Adelaide, Australia. Marked inter-fungal differences in hyphal ^{33}P transport over 10-35 mm distance to roots of *Medicago truncatula* confirmed our previous work, but this difference was not reflected in plant growth. The fungus transporting the smallest amount of ^{33}P also produced the largest plants containing most total P. The capacity for long-distance transport of P could be a fungal adaptation to an environment with a patchy distribution of available soil P. We have also shown that the intra specific variation in P transport by mycorrhizal fungi is rather small. This may reflect the clonal nature of arbuscular mycorrhizal fungi; the spread of a fungus during evolutionary time may have occurred without a major influence on fungal effectiveness in nutrient uptake.

2.2.2 The Effect of P Supply on the P Uptake Rate in Plants with or without Mycorrhiza

(I. Jakobsen)

An instantaneous supply of P to P-deficient plants resulted in the down-regulation of P uptake by roots of non-mycorrhizal plants. This was not observed in mycorrhizal plants where the highest uptake rate was also observed sooner than in non-mycorrhizal plants. These results do not support the hypothesis that mycorrhizal fungi reduce the root P uptake rate via feedback regulation.

2.2.3 Interaction between Micro Organisms with a Potential Use for Biocontrol and Mycorrhizal Fungi

(I. Jakobsen, S. Ravnskov)

Soil inoculation with micro-organisms, which can increase plant nutrient uptake and control plant diseases, represents a promising tool in sustainable agriculture. However, no organism should be released until its possible negative impact on other important functional groups of the soil ecosystem has been investigated. Such interactions have been studied in collaboration with The Royal Veterinary and Agricultural University for mycorrhizal fungi, saprophytic fungi and bacteria. A *Trichoderma* fungus, which is an effective biocontrol agent against several fungal plant pathogens, had no adverse effect on a mycorrhizal fungus, which on the other hand suppressed the biocontrol agent. Likewise, a plant-growth promoting *Pseudomonas* had no marked effect on a mycorrhizal fungus, which could negatively influence the growth and cultivation of the bacterium.

2.2.4 Molecular Studies of the Interaction between Mycorrhizal Fungi and Plants

(S. Burleigh, I. Jakobsen, J. Nielsen)

We have initiated molecular studies of the interaction between mycorrhiza and plants by identifying and characterizing differentially expressed genes both from the plant and the fungus. Two genes are currently characterized; one is involved in programmed cell death and the other in the transport of Zn. In addition we have initiated the study of the transport of P across plant/fungal membranes using already cloned plant P-transporters from other laboratories.

2.3 *In Vivo* NMR Technology to Study Transport during Symbiosis

(L. Rosendahl, H.Egsgaard, A.M. Scharff)

We have established a technology to study assimilation of symbiotically fixed nitrogen in intact legume root nodules by *in vivo* ^{15}N-NMR. Nanna Rasmussen has initiated a Ph.D. study entitled: Phosphate translocation in mycorrhizal fungi studied by ^{31}P NMR spectroscopy.

2.4 *Erysiphe graminis*/Barley Symbiosis

Genetic analyses and techniques in molecular biology are applied to identify avirulence genes and genes involved in the infection process of the parasitic symbiosis between *Erysiphe graminis* f.sp. *hordei* and barley.

2.4.1 A Map Based Cloning Approach to Isolate Avirulence Genes

(C. Pedersen, H. Giese, B. Wu)

We are using the map based cloning strategy to isolate avirulence genes. Both genetic and physical mapping of the powdery mildew fungus *Erysiphe graminis* f.sp. *hordei* genome has progressed rapidly in 1998. A genetic map comprising 285 markers and 7 avirulence genes has been established. The AFLP technique is now carried out on the sequencer using fluorescent markers and we have emphasized the integration of cDNA markers into the map. In collaboration with the Carlsberg Laboratory, an EST sequence database has been generated and we are using these sequences for the mapping. This type of marker can efficiently bridge the gap between maps generated from different *Erysiphe graminis* f.sp. *hordei* crosses to construct integrated maps. This is a central part of collaboration in an EU mobility program. The map distances are very high indicating that the powdery mildew fungus has a high recombination rate. A bacterial artificial chromosome (BAC) library estimated to cover the genome more than 10 times has been prepared. It will provide a suitable basis for physical mapping of the genome, cloning of avirulence genes and large-scale DNA sequencing.
The elicitor-receptor model is widely accepted in gene for gene resistance.

2.4.2 Isolation of Avirulence Genes by Phage Display

(P. Mouritzen)

According to the model, resistance is mediated by recognition of the fungal AVR protein (elicitor) by a receptor on the epidermal cell surface of resistant barley cultivars. Phage display technology provides a direct physical link between a protein displayed on the surface of a phage and the cDNA encoding this protein. We are attempting to use this system to clone avirulence genes and other fungal genes, encoding proteins involved in interactions with barley proteins. Two cDNA libraries have been constructed from barley epidermal strips infected with *Erysiphe graminis* f.sp. *hordei* spores. One library contains barley and *Erysiphe graminis* f.sp. *hordei* cDNAs from the stage of penetration of the epidermal cells by the fungal hyphae and the other barley and *Erysiphe graminis* f.sp. *hordei* cDNAs from the stage of fungal haustoria formation. In both libraries 1/3 of the cloned cDNAs represent *Erysiphe graminis* f.sp. *hordei* sequences. The cDNA libraries will be transferred to phage display vectors in order to display the encoded proteins on the surface of phages. These phage display libraries will be used in panning experiments on immobilized epidermis plasma membrane vesicles, obtained from resistant barley varieties. The panning procedure is under development. As a control we have used already characterized *Erysiphe graminis* f.sp. *hordei* genes to test the phage display vectors.

2.4.3 Isolation of Differentially Expressed Genes

(H. Giese, L. Baunsgaard, M. Grell)

The differential display technology has been used to identify genes that are important for the establishment of the *Erysiphe graminis* f.sp. *hordei* barley symbiosis. More than 30 cDNA fragments differentially expressed early in the infection process have been isolated and sequenced. Two of these have been further characterized. The differential expression pattern has been confirmed, using RT-PCR analysis. Full-length cDNA clones have been isolated. The first encodes a 172 aa transmembrane protein that is highly up-regulated at the time of haustorium formation (14-16 hours after infection). The second encodes a 335 aa protein enhanced at the time of spore germination (around 4 hours after infection) and throughout the stages of appressorium (10-12 hours after infection) and haustorium formation. Both proteins are down-regulated prior to the third day of development, when a second round of haustorium formation takes place.

2.4.4 Development of a Transformation System

(S.K. Christiansen, L.G. Jensen)

To verify and analyse the expression profile of identified fungal genes a transformation system is required. A stable transformation system for the powdery mildew fungus is currently under development using basta resistance. Transformation vectors have been constructed. We are now using a hand held gene gun to carry out *in planta* particle aided transformation of *Erysiphe graminis* f.sp. *hordei*. To distinguish genetically transformed fungi from their wild type relatives, a gene

originating from the jelly fish *Aequerea victoria* encoding a bioluminescent marker is used for the procedure. The gene-product is a protein that emits green fluorescence while excited with blue light. By applying this live marker in the transformation experiments, it is also possible to monitor the growth and development of fungi, expressing the foreign green fluorescent protein (GFP). Additionally the technique provides the basis for a procedure to select spores, carrying the GFP-gene in the genome and thereby being able to record the transmittance of the novel genetic trait to the following generations.

2.5 Marker Technology for *Tilletia caries*

(S.K. Christiansen)

The AFLP technology has been used to characterize different isolates of the seed borne pathogen *Tilletia caries*, causing the common bunt disease in cereals. A very low level of polymorphism was found between isolates from different Danish locations and from the Czech Republic. The genome of the fungus has been estimated to be very small, compared to other fungi.

2.6 Applied Chemistry

2.6.1 Methods for the Reduction of Organic Compounds from Gassifiers

(E. Larsen, H. Egsgaard, L. Frøsig)

A novel approach (De-TAR) for reduction of the content of organic compounds in condensates from, e.g. updraft gassifiers has been developed. The strategy is based on catalytic oxidation and has successfully been tested in the laboratory. The potential of the method is currently being evaluated with industrial partners. There is an increasing demand for characterization of organic compounds, possibly released from various materials as a result of thermal impact. A major effort is directed towards the elucidation of the underlying chemistry. We have for a number of years developed advanced analytical methods based on on-line measurements for this purpose.

2.6.2 Establishment of Mass Spectrometry for Peptide Analyses

(H. Egsgaard)

A mass spectrometric facility devoted to the analysis of peptides/proteins and other biomolecules has been installed in December 1998. The core of the system is a quadrupole ion storage mass spectrometer equipped with electrospray ionization including nano-spray. The instrument operates in the very low picomole range with a dynamic resolution on the order 7000. The nominal mass range is up to 4000u. An outstanding feature of the instrument is the MSn capabilities, which, due to the very low loss of ions during the collision activation, becomes a true analytical tool. Thus, MS4 to MS6 may routinely be performed on isolated substances, enabling a detailed mass spectrometric characterization of the compound in question. This instrumentation will be used to characterize proteins isolated from 2-D gels. Protein profiles are generated from different symbiotic systems and the proteom analyses will be used to identify proteins.

3 Plant Products and Recycling of Biomass

In 1998 this programme was established to develop plants with better quality by use of modern breeding technology. This included production of transgenic plants, particularly cereals, in order to create an overproduction of enzymes or to alter specifically the gene expression to modify the nutritional quality (phosphorus and nitrogen) or to modulate the polymer content like lignin. The growing public interest in the fact that our lives depend upon the availability of ever-diminishing reserves of petroleum has given momentum to the search for renewable resources as chemical raw materials. The carbohydrates potentially available from biomass waste products, such as wood chips and agricultural residues or purposely grown crops, represent one conceivable alternative.

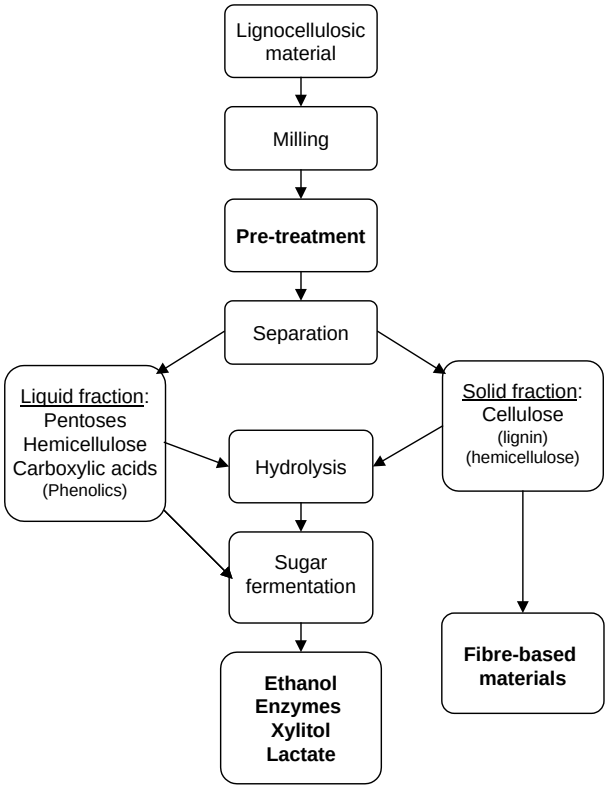

Figure 3.1.1 Process diagram for production of chemicals from renewable resources.

3.1 Production of Chemicals by Oxidative Hydrolysis of Biomass

(A.B. Thomsen, H. Klinke, A.S. Schmidt)

Wet oxidation is the process of treating material with water and air or oxygen at temperatures above 120°C. Recent work carried out in the programme of "Plant products and recycling of biomass" indicates that the process was an efficient and feasible way of fractionating low value biomass such as wheat straw, willow and other plant residue into hemicellulose, carboxylic acids and cellulose enriched fibres. The major advantages of the process are that: 1) it uses inexpensive chemicals - only oxygen and water; 2) the process is exothermic and heat recovery may be visualized; 3) it appears to be suitable for a wide range of biomass; 4) it increases the accessibility of cellulose to acid and enzymatic hydrolysis; and 5) the fibres can be used as reinforcement in polypropylene composites. After acid or enzymatic hydrolysis, the hemicellulose is transformed into fermentable xylose, which can be used as carbon source for fermentation of various products *e.g.* ethanol, xylitol and lactate as shown in Figure 3.1.1.

Reaction temperature and time were important parameters during wet oxidation pre-treatment (Schmidt and Thomsen 1998). Optimum reaction conditions for the fractionation (high solubilization and recovery of hemicellulose) of wheat straw varied with harvest year. The optimal reaction temperature ranged between 185 and 195°C and the reaction time between 10 and 15 minutes when treating 60 g/L straw (Schmidt and Thomsen 1998; Klinke *et al*. 1998a). Previously, the production of fermentation inhibitors, 2-furfural and 5-hydroxymethyl-2-furfural, from thermal sugar degradation could be avoided by adding small amounts of carbonate during wet oxidation.

Phenolic compounds from lignin degradation were formed at mg/L levels (Klinke *et al*. 1998a) with no or only limited effect on the ethanol fermentation process even at consi derably high concentration levels (Ahring *et al.* 1998; Klinke *et al*. 1998b).

The fibres derived from wet oxidation were very assessable for enzyme treatment yielding glucose. This was explained by two important factors: 1) oxidation and partly removal of lignin since steric factors caused by lignin tend to reduce the rate of hydrolysis; 2) degradation of the crystalinity of cellulose, which was difficult to hydrolyse without pre-treatment. Fourier Transform Infra-Red (FTIR) analysis of the fibre surface showed that more hydroxyl groups are found on the wet oxidized fibres and almost all ester bonds were removed (Schmidt *et al*. 1998). By cleaving acetate ester bonds, a significant amount of acetic acid was produced together with other carboxylic acids, which are common end products during wet oxidation (Thomsen 1998; Thomsen and Kilen 1998). The carboxylic acids may serve as direct nutrient source for biogas production whereby all

carbons would be utilized in the treated biomass. A new ethanol concept is presently being developed in collaboration with The Department of Biotechnology, The Technical University of Denmark, combining thermophilic and mesophilic fermentation of the sugars in wet oxidized biomass followed by subsequent biogas-production of the end products.

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3.2 Stress-Related Peroxidases
(B.K. Kristensen, S.K. Rasmussen)

The functions of the two stress-induced barley leaf peroxidases Prx7 and Prx8 have been studied. Prx8 was previously characterized as an extra-cellular pI 8.5 peroxidase. The powdery mildew fungus induces expression of Prx8 at early stages of fungal attack. The full-length cDNA encoding Prx7 was recently obtained and the protein was purified from barley coleoptiles and shown to be a pI 9.3 intracellular, putatively vacuolar peroxidase (Kristensen *et al*. in press). Prx7 accumulates more slowly than Prx8 following inoculation with powdery mildew conidia, but Prx7 is predominantly localized in the epidermis, while Prx8 is predominantly accumulating in the apoplast surrounding mesophyll cells. Functional assays, using Prx7 and Prx8 DNA constructions were performed in collaboration with the Department of Physiology at the Carlsberg Laboratory. Transient co-expression of Green Fluorescent Protein (GFP) and either Prx7 or Prx8 in barley leaf cells resulted in a decrease in the colonization rate of the powdery mildew fungus in comparison with the rate in GFP expressing control cells. Prx7 appeared to be more efficient in stopping the fungus than Prx8. Collaboration with the Department of Physiology continues in order to investigate the specificity of Prx7 and Prx8 mediated resistance. We continue to investigate the biochemical mechanism behind the increased resistance by analyses of genetically transformed barley plants engineered to over-express and abolish the expression of Prx7 and Prx8.

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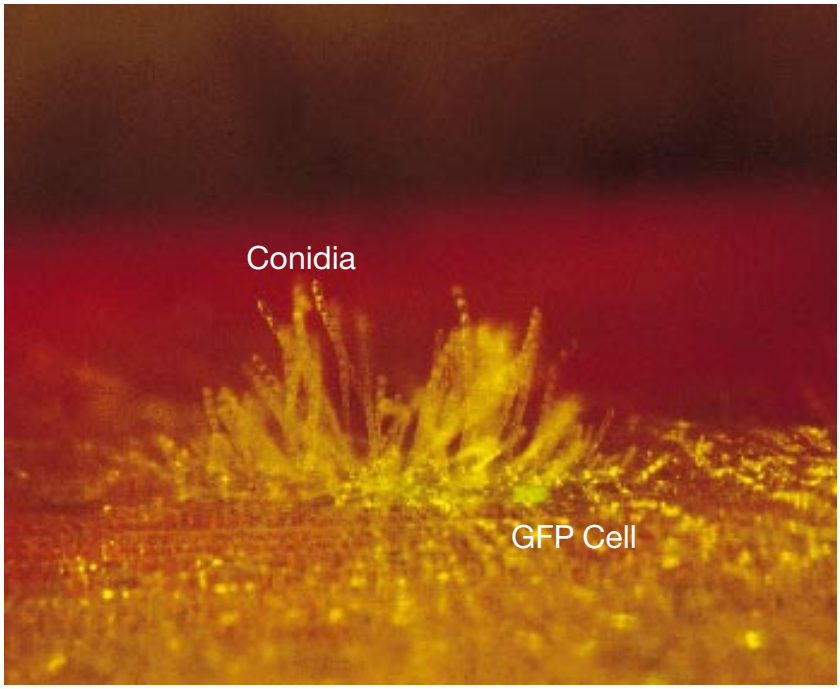

Figure 3.2.1 GFP used as in vivo transformation marker to assay the effects of Prx7 and Prx8 on colonization of barley leave cells by powdery mildew fungus.

3.3 Low Phytate Mutants in Barley Promise Improved Nutritional Quality and Lowered Environmental Risks

(F. Hatzack, K.S. Johansen, S.K. Rasmussen)

In cereal grains the majority of phosphorus is stored in the form of phytate (inositol hexakisphosphate) which unfortunately is only poorly digested by monogastric animals, like pigs and poultry. This situation has impact on environment and economy. The release of undigested phytate into rivers and streams increases the concentration of P, when phytate is finally digested by micro-organisms.

Economically, life stock keepers have to spend extra money to buy P-enriched fodder and/or phytase, which degrades phytate in the stomach of the animals.

One solution to the problems associated with phytate is the development of novel crops, which are low in phytate but rich in nutritionally available P.

Barley plants of this kind were first discovered at Risø by screening a large population of mutants and from the genetics it became clear that phytate synthesis is controlled by at least two different genes (Rasmussen and Hatzack 1998). Furthermore, by measuring phytate in two consecutive plant generations, it was found that phytate levels do not vary considerably, as being low in phytate (and rich in free P) is a stable inherited trait.

Almost normal development was observed in three mutants, derived from the malting barley 'Alexis' in field trials, conducted by Sejet Plant Breeding A/S.

Remarkably, one of these mutants contained only 5% of the wild type phytate level, suggesting that 'high-phytate' is not a requirement for normal plant growth.

The strong chelating capacity of phytate could lead to a loss of nutritionally important minerals when plants with low phytate levels are selected.

Atom absorption spectrometry analyses showed that Ca, Mg and Zn levels in low phytate mutants were similar to those found in wild types. Moreover, quantification of the total nitrogen present in kernels showed no significant differences between low phytate and wild type kernels. Taken together, this year's data made us confident that novel low phytate crops may be developed from our collection of barley mutants without jeopardizing other nutritionally important parameters like mineral and protein content.

References

Rasmussen, S.K., Hatzack, F. (1998) Identification of two low-phytate barley (*Hordeum vulgare* L.) grain mutants by TLC and genetic analysis, Hereditas 129:107-113.

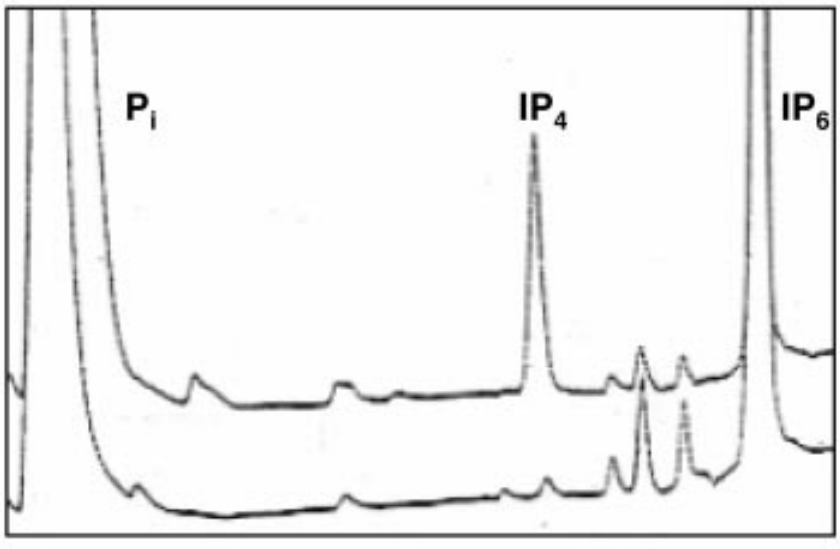

Figure 3.3.1 Metal dye detection MDD-HPLC analyses of low phytate barley mutant. The main difference between chromatograms from mutant (upper trace) and wildtype (lower trace) grain extracts is the presence of an inostol tetrakisphosphat (IP4) in the mutant.

4 DLF-Risø Biotechnology

The programme was initiated in 1998, through the establishment of the biotechnology consortium among DLF-TRIFOLIUM A/S, Research Division, and Risø National Laboratory. The programme is co-funded by The Danish Research Councils ('THOR programme). A major aim is to characterize and control key genes involved in the regulation of flowering in ryegrass (*Lolium perenne* L.), with the overall objective of developing transgenic high value grass plants, which are incapable of producing stems and flowers during grassland farming (*biological encapsulation*) and with highly improved quality for agronomic use.

Forage grass is by far the largest agricultural crop in the EU, occupying a total of 20 mill. ha in frequent rotation. Compared to other agricultural crops, the grass field has a very positive environmental profile due to limited use of pesticides, limited seepage of nutrients and the allowance of a large diversity of wild plant species, insects and animals. However, the value of grass as cattle fodder is limited by the fact that especially the grass stems contain high amounts of low digestible, mainly lignin-containing, compounds. A major breakthrough would be the development of ryegrass varieties that only grow vegetatively, without stem and flower tissue. Furthermore, controlled biological encapsulation technologies developed in this programme will provide methods to avoid dispersal of active transgenes in nature.

The programme employs 15 newly engaged people and started up in autumn 1998, when the group moved into new laboratory facilities at Risø National Laboratory. It encompasses a number of research activities, including 1) isolation and characterization of key genes involved in stem and flower formation, 2) development of novel conditional gene expression systems, 3) plant transformation systems, and 4) transgenic studies. The plant transformation activities are undertaken at DLF-TRIFOLIUM, Research Division, Store Heddinge.

4.1 Isolation and Characterization of Flowering Genes from Ryegrass, *Lolium perenne* L.

(C.H. Andersen, H. Richter, C.S. Jensen, K. Petersen)

One of the main goals of the programme is to identify ryegrass genes which are up or down regulated as a consequence of the induction to flowering. Different plant tissues and organs - leaves, roots, stems, meristems and flowers - are harvested at various time-points during vernalization (essential cold period in which *L. perenne* acquires the competence to flower) and secondary induction (increased temperature, long day), and expressed genes (mRNA) are isolated. Gene expression profiles are compared through the methods described below, and differentially expressed genes are isolated and further characterized. For these experiments, a total of 17,000 ryegrass meristems at different developmental stages are isolated.

For the isolation of genes that are up-regulated in one tissue compared to another, two different *subtractive hybridization* techniques have been implemented. In both methods the common 'non-interesting' genes are subtracted from the up-regulated target genes by hybridizing mRNA from induced plants to cDNA made from mRNA of control plants. Genes only expressed in the induced plants will not hybridize and these can subsequently be cloned and sequenced. Furthermore, we have developed an essential tool for the analysis of gene expression patterns in meristems: the method combines subtractive hybridization with a technique that enables the amplification of entire mRNA populations from very small amounts of plant material (< 5 mg).

Another method, ***D**ifferential **D**isplay **R**everse **T**ranscriptase **P**olymerase **C**hain **R**eaction*, (DDRT-PCR), is used for direct identification of differentially expressed genes.

Figure 4.1.1 Apex at the spikelet differentiation stage.

This very sensitive method identifies genes that are either induced or repressed in one plant/tissue relative to another. In addition, methods for the use of the DDRT-PCR technique for expression profiling of specific gene families, *e.g.* transcription factors, are being developed.

Using the genetic information of flowering genes from other plants we isolate the corresponding genes from ryegrass. The construction of ryegrass cDNA libraries is performed using specific tissues at various developmental time points and will provide a base to isolate full-length genes of interest. Expression patterns of the isolated flowering genes are analysed through time course studies involving both northern blotting and *in situ* hybridisation, and gene family size is analysed by Southern blotting hybridisation.

At present, more than 15 orthologs to flowering genes from other plant species, belonging to 7 different gene families, have been isolated from ryegrass. These include MADS box orthologs of the AP1 and Agamous subclasses, as well as genes showing homology to LEAFY, TFL and GA-MYB. The AP1 MADS box and LEAFY homologs have been characterised by Genomic Southern blotting and Northern blotting analysis.

4.2 Conditional Expression Systems for Plants
(T. Didion, M. Storgaard)

Plant science, both basic and applied, takes advantage of the use of transgenes to manipulate biological processes in transgenic plants. For certain applications, the expression of a gene of interest is desired only in particular tissues, at a particular period of the plant development or in a particular generation or progeny. Therefore, it will be necessary to develop and test a number of different systems for constitutive and/or regulated expression of transgenes in plants in general and especially in ryegrass.

One of those strategies we are working with is the isolation of 'tissue-specific' promoters to restrict the activity of the gene of interest to certain tissues. Other strategies currently under investigation are the development of chemically inducible expression systems and the development of chimeric transcription activators and promoters, which are expressed separately in different plant lines in order to keep them inactive at this stage. The expression of the gene of interest is only achieved by crossing these two plant lines thereby generating a hybrid plant with an activated expression of the transgene.

A number of such strategies have been adapted, e.g. a novel hybrid-based transactivation system which is currently under investigation in transgenic ryegrass and *Arabidopsis* plants.

4.3 Transformation of Ryegrass
(M. Folling)

The role of selected candidate genes in the control of floral induction is investigated by means of transgenic studies. The activities of potential activators of the floral induction are analysed through down-regulation of gene expression (anti-sense, co-suppression) in transgenic ryegrass and *Arabidopsis* plants, whereas potential repressors of the switch to flowering will be over-expressed in transgenic plants. The transgenic plants will be subjected to vernalization and analysed with respect to gene expression, capability and timing of flower formation and fertility of developed flowers.

Transformation of ryegrass is performed by direct gene transfer. We use two different systems routinely – particle bombardment with an inflow gun and PEG-mediated gene uptake by protoplast. In both systems, the source of target cells is embryogenic suspension cells initiated from either meristems or immature embryos. The protoplast system is highly efficient and a large number of transformants with a low copy number can be obtained. Particle bombardment is less efficient and usually gives rise to plants with multiple copies of the construct. Transformation of ryegrass with constructs containing candidate flowering genes has been initiated.

Figure 4.2.1 Production of transgenic ryegrass plants at DLF-TRIFOLIUM. Research Division, Store Heddinge.

5 Plant Genetics and Epidemiology

Our research has aimed at 1) increasing the knowledge about fungal disease resistance in plants by improving the availability of effective and durable resistance for plant breeding, 2) developing molecular techniques and markers for rapid and effective identification of genotypes, and 3) analysing introgression of transgenes from crops into wild relatives as part of an ecological risk assessment.

5.1 Disease Resistance and Population Dynamics of Pathogen Populations

(H. Østergård, L. Eriksen, R. Jørgensen, M. Lyngkjær)

The sexual stage of the fungus *Mycosphaerella graminicola* causing Septoria tritici blotch of wheat was found for the first time in Denmark. The sexual spores are seen inside the structure (ascus) in which they are produced (figure 5.1.1). A number of these structures each containing eight spores are produced inside a spherical fruit body (pseudothecium), which is embedded in the wheat leaf tissue. A model describing the population dynamics of the fungus was developed. This model suggested that under certain conditions sexual reproduction could significantly reduce the durability of resistance in wheat cultivars (Eriksen *et al.* 1998; Lyngkjær *et al.* 1998).

In collaboration with T. Carver, IGER, Wales, we have investigated the induction of cellular accessibility (susceptibility) and inaccessibility (resistance) to powdery mildew (*Blumeria graminis)*. The available evidence suggests that the induction in cereals is a general phenomenon expressed in living epidermal cells, which have survived prior attacks by the fungus. In all cases studied so far, the effects are dramatic in the fact that cells containing a haustorium, indicating an earlier successful *B. graminis* attack, are almost invariably penetrated by later attacks, while attacks on cells containing a papilla, indicating an earlier failed *B. graminis* attack, almost invariably fail. We suggest that the understanding of the cell biological basis of induced changes may reveal novel mechanisms of disease 'resistance' for exploitation through plant breeding. This could prove extremely valuable in the current situation where most single gene controlled resistances to cereal powdery mildew have been negated by the evolution of matching pathogen virulence, and increasing reliance is placed on quantitative resistance.

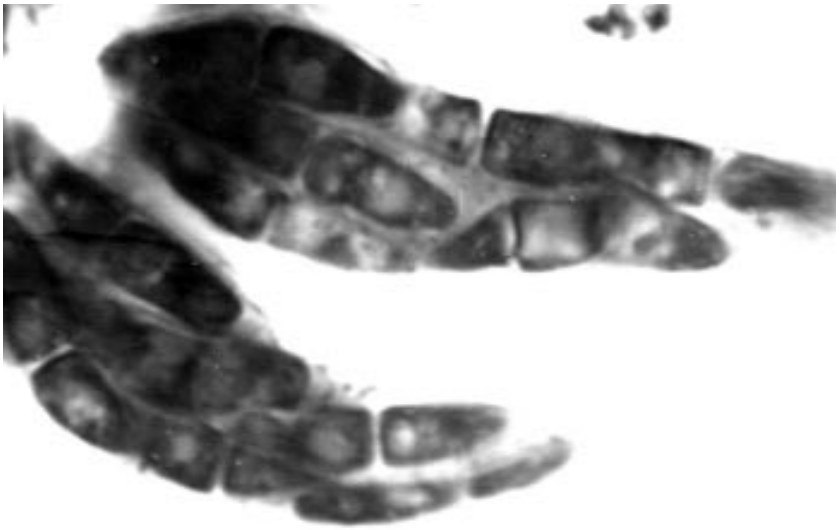

Figure 5.1.1 Sexual spores of Mycosphaerella graminicola.

A database in Access for handling experimental data on disease resistance collected within the project "Pesticide Free Cereals" of The Cereal Network, has been developed. This database also includes information from literature and other databases about disease resistance of a large collection of lines and varieties. Finally, the database is handling the seed storage facility at Risø.

References

Eriksen, L., Munk, L., Østergård, H. (1998) Modelling genes for aggressiveness in the *Mycosphaerella graminicola*/wheat pathosystem. In Resistance biology of agricultural crops. NJF-seminar nr. 302 Tune, Danmark, 25-27 November. Nordisk Jordbrugsforskning. In press.

Lyngkjær, M.F., Carver, T.L.W., Kunoh, H. (1998) Effects of previous *Erysiphe graminis* infection attempts on penetration resistance in barley epidermal cells. The 7th International Congress of Plant Pathology. Edinburgh, Scotland 9–16 August Abstract 1.9.5.

5.2 Molecular Markers in Barley

(A. Jahoor, J. Jensen, G. Backes, R.U. Johansen)

Molecular markers have a broad spectrum of application in the plant-breeding related research. In 1998 the main emphasis was on 1) the analysis of genetic diversity, 2) the localization of qualitative resistance genes against powdery mildew and leaf rust and 3) the localization of quantitative loci for malting quality, all of these in barley. The analysis of molecular markers was accelerated by the establishment of an automated sequencer that increased the through put of SSR (single sequence repeat) and AFLP (amplified fragment length polymorphism) markers. Barley has the advantage of being a kind of model plant for cereals by the relative simplicity of the genome, the large number of molecular markers available and the homoeology to the genomes of other cereals. In addition it is economically important for the Danish agriculture and industry.

The analysis of genetic diversity was performed in the framework of an EU-project, including sets of common genetic markers and barley lines for several participant institutions all over Europe. In this programme, SSR (single sequence repeat) markers were used in a set of 44 lines. The findings will be integrated in an analysis over the whole project.

Plant breeders are highly interested in obtaining new disease resistances against powdery mildew and leaf rust in barley. Therefore we are localizing genes for these resistances in several crosses, mainly with *Hordeum spontaneum*, the wild progenitor of our cultivated barley as resistant parent. F_2-outcomes from crosses for the localization of one unknown leaf rust gene and the powdery mildew resistance genes *Mli* and *Mlp* were analysed with the respective isolates. According to these tests, bulks with resistant and susceptible lines were arranged and analysed together with the respective parents by the help of SSR- and AFLP-markers in order to find markers, which are linked to the resistance and which will help subsequently to localize the resistances. As the result of this work, lines with the resistance genes will be available for the plant breeders, together with the respective genetic markers that simplify the integration of these resistance genes in future varieties. This will help to offer healthy varieties to the farmer, which allow decreasing usage of chemical plant protection.

Finally, loci for quantitative traits, relating malting quality were analysed in a cross between the varieties 'Alexis' and 'Regatta'. For quantitative traits, which can not be observed correctly with single plants or in single environments, genetic markers will be very important to improve the selection on the breeding stations. 'Alexis' has a very good malting quality and the systems of most breweries are tuned to the 'processing profile' of this barley variety. The DH-lines (double haploid lines) from this cross have been grown in field experiments in three locations by Danish plant breeders at Abed, Sejet and Pajberg. The seed from these experiments are now being analysed for a number of malting characters. QTL for the traits will later on be mapped on the barley chromosome in order to assist the breeders in developing advanced barley malting varieties. In our attempt to establish a linkage map of molecular markers in this cross, a number of AFLP (amplified fragment length polymorphism) and SSR (simple sequence repeat) markers in addition to the RFLP and RAPD markers earlier mapped were added. The linkage map has now about 130 markers and the main part of the markers are rather evenly distributed. Based on the map, we have in co-operation with H. Skinnes from Norway found QTL for resistance to scald caused by *Rhynchosporium secale* located on barley chromosome 3 and 6 (Jensen and Skinnes 1998).

References

Jensen, J., Skinnes, H. (1998) QTL for rynchosporium resistance in barley. The Cerial Net Work's annual meeting, Slagelse, Denmark. (Danish abstract available).

Figure 5.2.1 Genotyping of doubled haploid lines from the cross Alexis x Regatta by means of AFLP analysis.

5.3 Risk of Growing Transgenic Plants

(R.B. Jørgensen, F.I. Shim, M. Johannessen, L. Hansen, M. Pertl)

The development and widespread commercial use of transgenic crop plants have raised concerns that these plants might have adverse agronomic and ecological effects. Recent updates show that the number of field trials with transgenic crops conducted is approximately 25,000 (60 crops with 10 traits in 45 countries). The most common traits tested are herbicide tolerance, insect resistance, product quality and virus resistance. Our research focuses on gene flow from transgenic crops to wild populations of related plants. This is the first step in a sequence of events that can lead to increased weediness of the wild recipient or to invasion of recipients in new habitants.

Oilseed rape (*Brassica napus*) is known to hybridize and back-cross spontaneously with the weedy relative *B. rapa* (Jørgensen *et al.* 1998). However, to what extent plant competition and density affects the introgression process is unknown. To test this, mixed populations of oilseed rape *B. rapa* and their interspecific hybrid were established in the field: Six different proportions were analysed: each proportion representing two densities of plants (90 and 20 plants/m^2).

The flowering period of the three parental genotypes overlapped, but the peek flowering of *B. rapa* was before that of oilseed rape with the peek flowering of the F_1 hybrids being in between. The F_1 hybrids produced more flowers than the two pure species, but the fertility of the F_1 hybrid pollen was reduced to approximately 34% (90-95% for the pure species).

Offspring plants harvested from *B. rapa* mother plants have been identified as *B. rapa*, F_1 hybrids or back-cross plants by way of morp hology, DNA markers and a specific transgene present in the F_1 parent. The results show that 8-11% of the offspring plants was derived from back-crossings between *B. rapa* and F_1 hybrids grown in a 35:1 proportion. More results are in progress.

References

Jørgensen, R.B., Andersen, B. Hauser, T.P., Landbo, L., Mikkelsen, T., Østergård, H. (1998) Introgression of crop genes from oilseed rape *(Brassica napus)* to related wild species – an avenue for the escape of engineered genes. Acta Horticulturae 459: 211-217.

6 Biogeochemistry

The research in the Biogeochemistry Programme is focused on the occurrence, transport, turnover and effects of trace elements and organic micro contaminants in agricultural and forest ecosystems. Trace elements are followed from the soil or the atmosphere to the crop, and through the human food chain. The effects of air pollution and global change are studied, both at the plant and at the ecosystem level. Major emphasis is placed on the development of new methods and processes, which can form the basis of an environmental and sustainable plant production.

6.1 Trace Elements

6.1.1 Trace Elements in Agricultural and Horticultural Products

(V. Gundersen, S. Stürup, A. Bibak)

Elemental profiles were studied in potatoes cultivated under different fertilizer and N-levels. The objective of the study, which was supported financially by the Danish Food Technology and Development Program (FØTEK), was to evaluate the elemental concentrations in potatoes (*Solanum tuberosum*, Folva) affected by application of three levels of N fertilization by pig slurry or calcium ammonium nitrate.

The experimental field was located at the Risø National Laboratory experimental farm, Dyskærgaard. It was cultivated and fertilized for more than 50 years before the experiments were started in the spring of 1995. The field was divided into 6 plots. 3 plots were fertilized with pig slurry and 3 plots were fertilized with calcium ammonium nitrate using the three N-levels: 0 kg, 60 kg, and 120 kg N/ha for both types of fertilizer.
From each plot 10 samples (5 tubers from each of 10 plants) were sampled for analysis. Pure pulp from the potato tubers was analysed with every necessary precaution against contamination by multi-elemental HR-ICPMS analysis.

The elements analysed were Ag, Al, Au, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Dy, Er, Fe, Ga, Gd, Ho, In, Ir, La, Lu, Mn, Mo, Nb, Nd, P, Pb, Pd, Pr, Pt, Rb, Re, Rh, Ru, Sb, Sc, Sm, Sn, Sr, Ta, Tb, Th, Ti, Tl, Tm, U, V, Y, Yb, and Zn.

The overall effects of three levels of N-fertilization on elemental concentrations of potato in each field site are evaluated by the use of Discriminant Partial least squares regression (PLS): The data used in the regressions are range normalized. The score plots are shown in Figures 6.1.1.1 and 6.1.1.2.

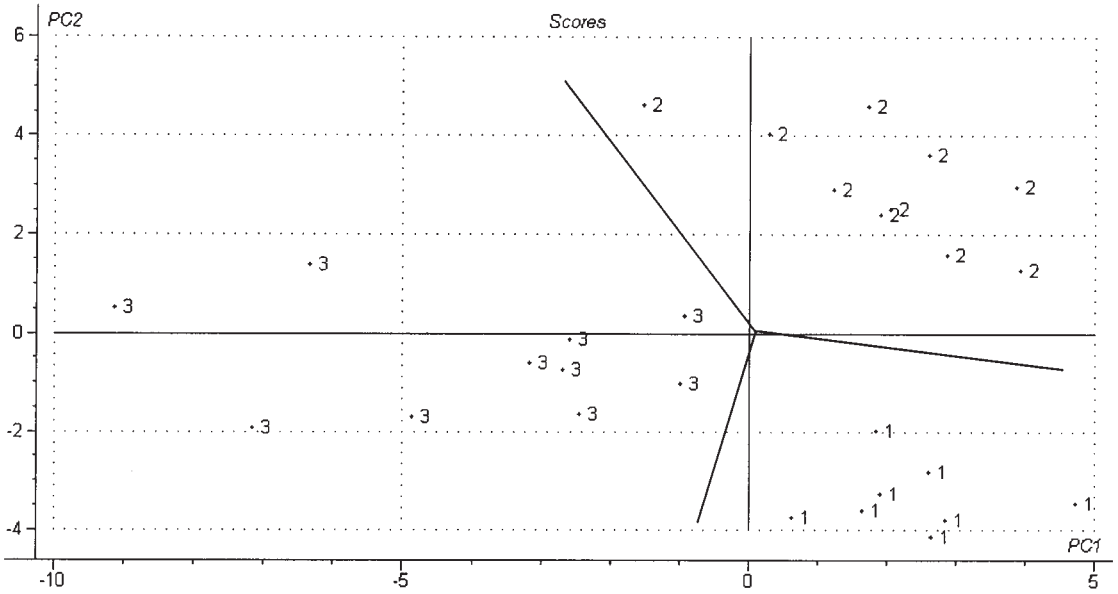

Figure 6.1.1 Score plot of the PLS regression for potatoes fertilized with calcium ammonium nitrate. PC1 explains 24% and PC2 15% of the variation in X. The lines in the plot incicate the 3 level of N-fertilization trends to split in the groups.

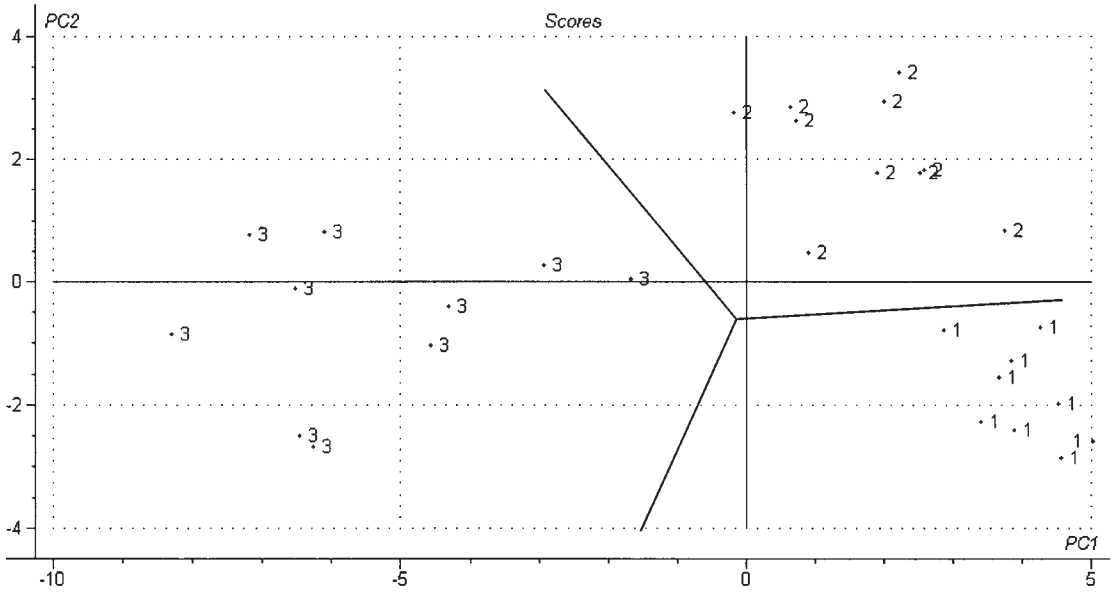

Figure 6.1.2 Score plot of the PLS regression for potatoes fertilized with pig slurry. PC1 explains 35% and PC2 7% of the variation in X. The lines in the plot incicate the levels of N-fertilization which end to split in the groups.

The score plots show that 0 kg N/ha 1), 60 kg N/ha 2), and 120 kg N/ha 3) split the samples in 3 groups separated by the 3 lines in the figures.

Analysis of variance (One-Way ANOVA and Duncan Multiple Range) indicates that the elemental profiles at each N-level show a clear difference between pig slurry and calcium ammonium nitrate. The elemental profiles are similar for potatoes cultivated with the same fertilizer and at the same N-level but different with different fertilizer and N-level.

6.1.2 Sediment Dating and Geochemistry

(H. Kunzendorf)

The Gamma Dating Centre (GDC) continued lead-210 dating of sediment cores supplied from both Danish and Nordic institutions. Major customers were the Swedish Environmental Research Institute and universities in Denmark and Norway. Participation in the regional seas research project BASYS (funded by EU, MAST3) continued in 1998. The work has been focused on lead-210 dating and on the paleoenvironmental signals in sediments from selected deep basins of the Baltic Sea (Bornholm Basin, Gotland Basin and North Central Basin).

Geo-chemical profiles from the Gotland Basin suggest a natural cyclic sedimentation pattern during the past 2000 years, because periods lasting about 270 years with enhanced salt water inflow from the North Sea were followed by periods lasting about 300 years with relatively stable inflow. Furthermore, the systematic analyses at Risø suggest that by being involved in the nitrogen-fixing of blue-green algae the trace element molybdenum is proposed to be an important indicator of past and present algal blooming periods in the central Baltic.

6.2 Organic Micro Contaminants

6.2.1 Plant Uptake of LAS and DEHP from Sewage Sludge

(C. Grøn, F. Laturnus, G.K. Mortensen)

In 1998 a greenhouse study was carried out for the Danish Environmental Protection Agency on the uptake in barley and carrot of the substances di(2-ethylhexyl)phthalate (DEHP) and linear alkylbenzenesulfonates (LAS) from sewage sludge. The study was in collaboration with the Programme for Plant Ecosystems and Nutrient Cycling, Risø. LAS was found in green barley plants, whereas DEHP was found in barley plants as well as in carrots and carrot tops. The content of LAS in the barley plants could not be related to the concentrations in the soil/sludge mixtures, but was rather attributed to indoor air and dust deposition. The only exception from this was elevated concentrations of LAS in barley roots at high sludge concentrations. For DEHP, concentrations in barley were varying independently of the DEHP concentrations in the soil/sludge mixtures, and indoor air deposition is therefore considered the origin of the DEHP in the green barley. For carrots, the DEHP concentrations were higher than in barley, with the highest concentrations in the peel of the carrots. The DEHP contents in carrots were not related to the concentrations in the soil/sludge mixtures. An indication of translocation of LAS or DEHP from roots to leaves was not found.

Overall, the study showed that LAS and DEHP were not taken up from sewage sludge amended soil by barley and carrots. The study also showed that the models used in risk

assessment are not yet developed to yield reliable predictions of the risk associated with organic contaminant uptake in crop plants from soil.

6.2.2 Polycyclic Aromatic Compounds - PAC

(T. Nielsen, A. Feilberg, C. Helweg, M.W.B. Poulsen)

In order to study the soil sorption of PAC, two different humic acids (HA) and a fulvic acid (FA) were chemically immobilized to a HPLC silica column material. The immobilization was performed by binding amino groups in HA/FA to the free aldehyde group in glutardialdehyde attached to the silica. The retention factors of 45 polycyclic aromatic compounds (PAC) to the columns were determined by HPLC. The comparison shows that the technique can be applied to studying the sorption of organic compounds to humic substances. The advantage of the technique is that a large number of compounds can easily be studied. The retention or sorption of the compounds increased with the size of the molecules and the number of lipophilic substituents. Polar groups caused the retention to decrease. The retention of the PAC appeared to be strongly affected by the size of the humic substances, but the content of aromatic carbon and/or the O/C ratio may also contribute. The relative effect of the size of the humic acid did not appear to be larger for the hydrophobic PAH than for the polar PAC.

Photodegradation is the dominant pathway for genotoxic PAC associated with atmospheric particles. Understanding the factors that influence the photodegradation rate is therefore an important issue in relation to the distribution of these anthropogenic pollutants. The focus has been on the strongly mutagenic nitro-PAHs, for which the photodegradation on soot particles has been known to occur via a complex unknown mechanism. A series of experiments have been conducted in order to elucidate the influence of aerosol chemical composition on the photodegradation rate. It has been clearly demonstrated that the decay is accelerated by the presence of certain compound groups including polycyclic aromatic ketones and quinones as well as substituted phenols and benzaldehydes.

Nitro-PAHs are present in ambient air both as a result of direct emissions and due to transformation of native PAHs, but the relative importance of these processes is uncertain. The occurrence of nitro-PAHs is currently being studied both in a semi-rural area and in the centre of a large city (Copenhagen) in order to understand factors that influence the composition and concentration levels. A GC-MS-MS method, which can measure nitro-PAHs with sufficient selectivity and sensitivity, has been developed. Some of the results obtained so far show that at both sampling sites the relative levels of those nitro-PAHs formed in the troposphere are strongly increased during long range transport of polluted air masses from the continent.

6.3 Effects of Air Pollution and Global Change

6.3.1 Atmospheric Degradation of Anthropogenic Molecules

(O.J. Nielsen, J. Sehested, J. Platz)

To assess the environmental impact of the release of a chemical compound into the atmosphere there are several issues that need to be considered. The first step is to determine its atmospheric lifetime. It determines the geographical extent of the possible direct environmental impact. To calculate the atmospheric lifetime we need information on the kinetics of its reaction with key atmospheric trace species such as OH and NO_3 radicals and O_3, the rate of its photolysis, its solubility and hence propensity towards wet deposition, and finally its rate of dry deposition. Once the lifetime of the pollutant has been established, the next step is to determine the degradation products and intermediates of its atmospheric oxidation, and to assess whether they pose any environmental threat. The work focussed on three areas: hydrofluoroethers (HFEs), aromatics and oxygenates as alternative fuels and fuel additives. All the work was done as collaborative studies with Ford Motor Company and several European research institutions. The overall aim was to provide the scientific basis for strategies for emission control. In 1998 we studied HFE-7200 ($C_4F_9OC_2H_5$), $CF_3CH_2OCH_2CF_3$, 2,3-butadione, acetone, trioxane, acetaldehyde, the phenyl radical, the phenoxy radical and the reaction of Cl atoms with benzene.

6.3.2 Effects of Global Change on Forest and Agro Ecosystems

(L. Rasmussen, C. Beier)

To evaluate the possible effects of climatic change on boreal forest ecosystems, both atmospheric CO_2 and air temperature were increased (CO_2 from 360 to 560 ppmv, temperature from3-5°C above ambient) at a forested catchment in southern Norway. The project is an international, co-operative research project called CLIMEX (Climate Change Experiments) located in a mountainous pine-birch forest (*Pinus silvestris, Betula pubescens*) at an elevation of 300 m above sea level. The entire catchment (860 m^2) is enclosed within a transparent greenhouse. Within 3 years, soil nitrogen mineralization has increased and the growing season has been prolonged, relative to the control area. This has helped to sustain an increase in forest floor plant growth, relative to the control and has also promoted increased nitrogen export in stream water. Photosynthetic capacity and carbon-nitrogen ratio of new leaves of most plant species did not change, and no significant effects on tree growth were observed.

The SOROFLUX project (Effects of land use and organic waste application on carbon and nitrogen fluxes) was implemented under the Danish Strategic Environmental Research Programme 1997-2000 (Centre for Sustainable Land Use and Management of Contaminants, Carbon and Nitrogen) as an extended national contribution to the EU projects EUROFLUX, EXAMINE and FOREXNOX. The project is in collaboration with University of Copenhagen, The Royal Veterinary and Agricultural University and University of Aarhus. The main

objectives of the project are to quantify and compare the gaseous and water mediated fluxes of N and C compounds in forest (beech) and agro (barley) ecosystems with and without accelerated input of N and C in the form of sewage sludge. The project provides quantification of the net ecosystem fluxes of NO, NO_2, NH_3, N_2O, HNO_3 (gaseous), CO_2, CH_4, H_2O and water mediated inorganic and organic N and C compounds. Measurements of the fluxes of the gaseous compounds are performed with advanced eddy-correlation technique, the gradient technique, the relaxed eddy-accumulation (REA) technique and dynamic chambers. Water mediated fluxes encompass rain, throughfall, stem-flow and leaching from the root zone. The results obtained so far show great variation in fluxes of the different C and N compounds both in time (day/year) and space (forest/fields). From Risø the following researchers are involved: L. Rasmussen, C. Beier, T. Mikkelsen, K. Pilegaard, P. Ambus, N.O. Jensen, P. Hummelshøj.

7 Plant Ecosystems and Nutrient Cycling Programme

The two most important events in the programme in 1998 are the establishing of CONFIRM and CLIMOOR.

7.1 CONFIRM

(P. Ambus, E.S. Jensen)

In 1997 the National Research Councils Instrument Centre Programme provided funding for CONFIRM (Centre for **Con**tinuos **F**low **I**sotope **R**atio **M**ass Spectrometry), which was established during 1998. The objectives of CONFIRM are a) to improve the possibilities for Danish environmental, ecological and agricultural scientists to use continuos flow isotope ratio mass spectrometry, b) to provide a forum for interdisciplinary research using stable isotope methodologies, and c) to educate masters and Ph.D.'s within environmental, ecological and agricultural sciences which are familiar with modern continuos flow isotope ratio mass spectrometry. The new instrumentation includes a Finnigan MAT Stable Isotope Ratio Mass Spectrometer interfaced to three different preparation systems: 1) an elemental analyser with options for simultaneous measurements of ^{15}N and ^{13}C in solid samples, *viz.* soil, plant or animal tissue; 2) GC-combustion interface, and 3) a trace gas preparation and concentration unit for isotopic characterization of atmospheric N_2O and CH_4 at ambient concentrations.

Research and educational institutes and companies that co-operate within CONFIRM include Danish Institute of Plant and Soil Science, Danish Forest and Landscape Research Institute, The Royal Veterinary and Agricultural University, Technical University of Denmark, University of Copenhagen, DANISCO Seed, and KEMIRA Denmark A/S.

7.2 CLIMOOR

(C. Beier)

CLIMOOR (**Cli**mate driven changes in the functioning of heath and **moor**land ecosystems) is an EU financed project comprising Denmark (co-ordinator), Holland, Wales, and Spain. The aim is to study the effect of a possible change in temperature and precipitation as an effect of increased emission of greenhouse gasses. On an open slope in Mols Bjerge (see photo below) three sites of 20 square metres are equipped with a system of shielding and curtains making it possible to create artificial simulation of the effects of global warming by heating the ground and reducing the exposure to rainfall.

By studying various parameters, such as temperature, species composition, and carbon fluxes it is possible to establish how the ecosystems will adapt to climate changes including which organisms will survive and which will die, and how warming will change the turn over of organic matter.

7.3 FOREXNOX

(T. Mikkelsen, K. Pilegaard, C. Beier)

Two other EU-projects were completed in 1998: FOREXNOX investigated European forests as a source of nitrogen oxides. It was found that forests often act as sources of NO_2 due to the reaction between soil emitted NO and O_3 in the trunk-space. It was also found that the N-affected European forests had a much larger NO emission than temperate forests in general. The increased emission of NO has a significant influence on O_3 budgets, and may thus affect the estimates of crop damage from O_3. EUROFLUX, which investigates CO_2 exchange over European forests, was also completed in 1998. The Danish EUROFLUX site (Ll. Bøgeskov) showed an average net uptake of 150g C m^{-2} yr^{-1}, with a large annual variation.

Figure 7.2.1 Effects of climatic changes tested at Mols Bjerge.

Some of the measurements started within the above mentioned projects are continued in the SMP-II financed SOROFLUX-projects dealing with circulation of C and N in a forest and in an agricultural system. The CO_2 and water vapour flux of single leaves is measured non-destructively by using a special cuvette system, and the xylem sap flow of the trees is measured using small probes. The canopy scale flux measurements have shown large differences between the forest and the agricultural system. A special part of the project deals with continuous measurements of the exchange of CO_2, O_3, NO and NO_2 at the soil-atmosphere interface. Measurements will be continued through the year 2000. The research is a co-work with the research programme for Biogeochemistry, Risø.

7.4 Organic Polutants
(L. Kure, P. Ambus, E.S. Jensen)

A project under the Centre for Sustainable Land Use and management of Contaminants, Carbon and Nitrogen aims to investigate which toxic organic contaminants are taken up through plant roots and subsequently translocated into leaves. We have developed a test system, in which young plants of different species are grown in nutrient solution. ^{14}C-labelled contaminants typically associated with sludge (LAS, 4-NP, DEHP, 1,4-dichlorobenzene, Pyrene, diethylglycol p-nonylphenylether) are added in solution and the ^{14}C activity in different parts of the plants are detected after exposure for up to four days. In 1998 we have tested the compounds on barley and rape, but will continue with other species in 1999. Preliminary results reveal large species- and compound differences. LAS *e.g.* is readily taken up and translocated to leaves by rape, but accumulates only in roots of barley, whereas 1,4-dichlorobenzene is taken up and translocated in barley but to a much lesser extent in rape.

7.5 Precision Farming
(G. Gissel-Nielsen, R. Jørgensen)

Ultimo 1998 the Danish Directorate for Development in Agriculture and Fishery has granted a 5 year project on Precision Farming, which makes it possible for us to expand our research on site specific fertilization to comprehend development of sensors and sensor systems. The Department for Optics and Fluid Dynamics, Risø, is involved in this work, and it is closely correlated with projects at the RVAU and with DIAS. Further, the directorate sponsors our project on genetically background for phosphorus uptake into barley.

7.6 Organic Farming
(H.H. Nielsen, P. Ambus, E.S. Jensen)

Under the programme of Organic Farming the above mentioned directorate sponsors our project on competitive interactions, resource use, and nitrogen dynamics in annual intercrops in low-input cropping systems. Earlier studies show a complementary use of N sources in pea-barley intercrops where pea is forced to rely on N_2-fixation because barley is more competitive for soil inorganic N. Field studies including soil water manipulation show that barley sole crop yield is maintained in the intercrop, independent of water supply whereas pea intercrop growth is considerably reduced.

Furthermore, weeds suppress pea rather than barley growth, possibly due to a rapid early weed growth and N uptake at a time when pea seedling emergence and formation of the first root nodules is not fully established. Improved knowledge of better pea performance in intercropping systems seems to be the basis for increased N input from symbiotic nitrogen fixation in the agro-ecosystem.

8 Other Activities

8.1 Dosimetry and Industrial Irradiation

(A. Miller)

8.1.1 Risø High Dose Reference Laboratory (HDRL).

The accreditation of Risø High Dose Reference Laboratory by DANAK (Danish Accreditation Scheme), registration number 266 continued through 1998. During the year 79 reports and 60 certificates were issued.

The services have expanded during the recent year, as shown by the redoubling of reports and certificates compared to last year. All services run without trouble, even in spite of the fact that the staff has been reduced because of the organizational changes within the Department. The 10 MeV accelerator is now hired to a private firm, LR Plast A/S, who operates the facility, but the HDRL has access to the use of the accelerator for calibration purposes and for training courses.

In November 1998 the 10 kCi facility was closed and decommissioned. The sources were transferred to Gammacell I in building 313. The dose rate was calibrated by transfer dosimeters from NPL, and Gammacell I is now operational again as the reference source of HDRL.

Polystyrene calorimeters for use at 2-3 MeV electron accelerators were constructed and are now in use at a commercial accelerator facility that operates at an energy of 2-2.5 MeV.

8.1.2 Dosimetry for Radiation Sterilization

The EU project on "Dosimetry for Radiation Sterilization" (STM4-CT96-2077) has continued with a second intercomparison with most of the European irradiation facilities as participants. The data are still being analyzed.

8.1.3 Accreditation for High Dose Measurement

The EU project on "Accreditation for High Dose Measurement" (IC15-CT96-0824) has continued with a workshop for the participants at NPL, UK. Fellowships planned for 1998 have been postponed to 1999.

8.1.4 Training Cources

Two training courses on Validation and Process Control of Electron Beam Sterilization were organized with 16 participants from 8 countries.

8.2 The sixth International Symposium on Genetics and Molecular Biology of Plant Nutrition held in Elsinore, Denmark from August 17-21, 1998

(A. Jensen)

The symposium was organized by Risø National Laboratory in the year of its 40th anniversary. The 98 participants represented 23 countries and 80 scientific contributions with 43 oral and 37 poster presentations.
The symposium addressed the molecular mechanisms, physiology and genetic regulation of plant nutrition. The Symposium brought together scientists from a range of different disciplines to exchange information and ideas about the molecular biology of gene systems, which influence and regulate mineral nutrition of plants.
The symposium emphasised:

- Solidifying the bridge between molecular biology, applied genetics, plant nutrition andplantbreeding.
- The development of methodology to improve the efficiency and effectiveness of nutrition of plants
- Quality of plant products.

With sessions on: Nitrogen; Phosphorous; Micro Nutrients; Symbiosis; Membranes; Stress; Heavy Metals and Plant Breeding.

In comparison with the previous conferences in this series more emphasis was placed on use of molecular techniques in delineating physiological mechanisms and processes, and in identifying genetic markers, genes, gene expression and regulation. Significant progress was reported in exploitation of molecular genetic markers and other biotechnologies in breeding programmes.

I wish to thank the contributors for the high scientific standard of their presentations and for thorough preparation of their manuscripts. The serious attempts to meet the editorial standards and to satisfy the wishes and suggestions of the referees are highly appreciated.

We are grateful to the International Council of Genetics and Molecular Biology of Plant Nutrition and members of the Organising Committee for their generous support and co-operation, from the planning of the scientific programme to arranging the symposium. Members of the International Council: N. El Bassam, B. Berkasem, K. G. Briggs, G. Ferrari, T. Fujiwara, R. R. Duncan, J. Dunlop, R. D. Graham, W. J. Horst, P. G. C. Kuiper, B. C. Loughman, Y. Masaoka, P. J. Randall, D. W. Rains, V. Römheld, P. N. Takkar and R. M. Welch.

The Organizing Committee, H. Giese, K. Hjortsholm, P. B. Holm, A. Jensen, G. Gissel-Nielsen and J. Schjørring, express its gratitude to Risø National Laboratory, the technical staff at The Plant Biology and Biogeochemistry Department and other people who helped to make the symposium run smoothly. We thank the Danish Veterinary- and Agricultural Research Council, Norsk Hydro Ltd, KEMIRA Ltd, ENERO and Risø National Laboratory for financial support.

Figure 8.2. 1 The picture shows approximately half of the participants in front of the congress centre, LO-skolen in Elsinore.

9 Special Facilities

9.1 The RIMI Field Station

(K. Pilegaard)

Risø Integrated Environmental Project (RIMI) is an interdisciplinary research project aimed at the study of pathways, processes and effects of anthropogenically and biogenically derived nitrogen compounds in the terrestrial environment. Nitrogen compounds emitted to the atmosphere play an important role in changes of the atmospheric composition and oxidation capacity, deposition of nutrients and acidification of ecosystems, and there is a substantial need for more precise information on both qualitative and quantitative aspects of the problem.

The integrated project follows several of the recommendations from an international evaluation of Danish environmental research. The project combines research from different disciplines such as atmospheric transport and chemistry, plant physiology, soil biology, biochemistry and geochemistry.

The field station is central in the project. It is situated in a rural area 2 km east of Risø National Laboratory. The area is a conventional Danish agricultural area. The field station is comprised of a 10 m meteorological mast placed in the middle of a field and an instrument hut placed at the margin of the field. The mast and the hut is connected with underground wires for power supply and data transfer. Continuous meteorological measurements are carried out at the site and it serves as a reference station for air pollution monitoring in Copenhagen by the National Environmental Research Institute. In 1998 a special experiment was carried out at the station concerning measurements of ammonia fluxes with a new technique called relaxed eddy-accumulation (REA).

The project is a joint effort with the programme "Atmospheric Transport and Exchange" in the Wind Energy and Atmospheric Physics Department, Risø.

9.2 Risø Environmental Risk Assessment Facility, RERAF

(K. Nilsson)

During 1998 all six growth chambers in RERAF have been used for several projects involving an array of plants including rapeseed, oat, pea, carrot, maize and tomatoes. Two of the growth chambers are equipped with CO_2-controlling systems, and these were in 1998 primarily used to study the influence of elevated levels of CO_2 on the growth of oak and beach trees.

Each of the growth chambers is equipped with its own individual climate control system, which controls light, temperature, humidity and in addition, can simulate the diurnal cycle for all three parameters in a near natural manner. This set-up very dependable.

Two of the growth chambers were classified early in the year for work with gene-modified plants.

9.3 Dyskærgård, the Experimental Farm

(F. Hasselbalch)

The climate in the autumn of 1997 was dry and sunny and the winter of 1998 was mild and humid so the use of nitrogen was as usual. The winter crops were winter barley (13% of area), winter wheat (9% of area) and winter oilseed rape (13% of area). In late spring a period of strong frost resulted in late sowing of the spring crops, including spring barley (30% of area) and peas (4% of area). The summer was wet and cold so fungicides had to be applied on the cereals. The yields were a little above average, highest for oilseed rape and lowest for peas. In the herd of Hereford cattle with 25 mother animals, 22 calves were born.

The distribution of crops and 17 field experiments (*e.g.* nitrogen application and utilisation, weed and disease control, crop-weed competition, organic plant production, selection for disease resistance, biomass crops on set a side areas) were summarised graphically with a short description of each experiment in a leaflet entitled "Risø Markplan 1998".

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Backes, G., Kicherer, S., Baum, M., Jahoor, A. Localising QTLs for resistance against powdery mildew and leaf rust in barley. Cerialienetværkets årsmøde, Slagelse, Denmark. (Abstract available).

Backes, G. The value of QTLs in pre-breeding. Nordic Workshop on Pre-breeding, Landskrona, Sweden 15 September.

Backes, G. Analysis of quantitative resistance loci in barley, NJF Seminar 302, Tune, Denmark 25-27 November.

Beier, C. Global warming, N deposition and N saturation: The CLIMEX project and extrapolation using the MERLIN model. Conference on nitrogen, the confer-N-s, Nordwijkerhout, The Netherlands, 23-27 March.

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Giese, H. Debatindlæg om forskeres rolle. Danmarks Forskningsråds konference om Sektorforskningens rolle og rammebetingelser, Forsikringens Hus, Copenhagen, Denmark, 30 April. (In Danish).

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Grell, M.N. Isolation and characterization of *Erysiphe graminis* f. sp. *hordei* infection genes. Seminar at University of Copenhagen, Denmark, 27 May.

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Hatzack, F., Rasmussen, S.K. Automated HPLC Analysis of Phytate: a Tool for Screening Programs and Metabolic Studies. Cerealienetværkets årsmøde, Slagelse, Denmark, 5-6 November.

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Jahoor, A. Powdery mildew resistance genes in barley, Carlsberg Laboratory, Denmark, 2 December.

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Johansen, K.S., Hatzack, F., Rasmussen, S.K. Lowphytate mutants and transgenic crops with high phytase activity - two strategies to improve availability of phosphate from cereal grains. Genetics and Molecular Biology of Plant Nutrition, Helsingør, Denmark, 17-21 August.

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Jørgensen, R.B. Genetisk modificerede planter – en risiko for miljøet? Seminar om genetisk, modificerede organismer og det økologiske jordbrugs forbud, Rødding Højskole, Denmark, 1 March (Abstract available).

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Lyngkjær, M.F. Induced accessibility and inaccessibility in barley epidermal cells by a compatible *E. graminis* f.sp. *hordei* isolate. 16th Nordic post graduate course in plant pathology, resistance to plant pathogens, Norway, 18-25 April.

Miller, A. Bestrålingsanlæg og processer. Seminar om sterilisation af medicinsk udstyr. LR Plast, Glostrup, 12 March. (In Danish).

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Møller, M.G. Isolering og karakterisering af gener involveret i syntese og nedbrydning af asparagin. Forskningsdag for Planteforædling og Bioteknologi 1998, University of Odense, Denmark, 7 January. (In Danish).

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Nielsen, O.J., Sehested, J., Egsgaard, H., Wallington, T.J. Atmospheric and combustion chemistry of oxygenated fuels and fuel additives. Meeting at the Technical University of Denmark, 9 January.

Nielsen, O.J. Atmospheric Chemistry – Two cases. Roskilde University, Denmark, 30 October.

Nielsen, T. PAH and other PACs in the atmosphere. Annual Meeting of the Danish Chemical Society. University of Odense, Denmark. 11 June.

Nielsen, T., Gissel-Nielsen, G. Phytotoxicity and sorption of N-PAC compounds. Winter Symposium on pollution of groundwater. Danish Academy of Technical Science. Vingsted, Denmark. 10-11 March.

Pedersen, C. Genetic and physical mapping of the powdery mildew fungus. Fungal Genomics, Athens, USA, 26-27 March.

Pilegaard, K., Hummelshøj, P., Jensen, N.O. Exchange of No_x and O_3 at a beech forest floor. EUROTRAC-2 symposium, Garmish-Partenkirchen, Germany, 23-27 March.

Pilegaard, K., Hummelshøj, P., Jensen, N.O., Chen, Z. Contrasting the results of the two first seasons of continuous CO_2 eddy-flux measurements over a Danish beech forest. – FLUXNET workshop, Polson, USA, 3-5 June.

Pilegaard, K. Biosphere-atmosphere exchange research at Risø. Lund, Sweden, 3 December.

Pilegaard, K. Two years of CO_2 flux measurements over beech. The Danish contribution to EUROFLUX, Risø, 6 October.

Platz, J., Sehested, J., Nielsen, O.J., Wallington, T.J., Ball, J.C., Hurley, M.D., Straccia, A.M., Schneider, W.F. Atmsopheric chemistry of the phenoxy radical, C_6H_5O, UV spectrum and kinetics of its reaction with NO, NO_2 and O_2. 15th International Symposium on Gas Kinetics, Bilbao, Spain, 6-10 September.

Poulsen, M., Feilberg, A., Nielsen, T. Analysis of nitro-PAH in the atmosphere. Annual Meeting of the Danish Chemical Society. University of Odense. 11 June.

Rasmussen, N. Nutrient transport by AM mycelia. NMR studies of P metabolism and translocation. Workshop on Arbuscular Mycorrhizas in Sustainable Soil-Plant Systems, COST-Action 821, Uppsala, Sweden, 3-4 July.

Rasmussen, L. Tree responses to increased CO_2 and temperature studied by experimental manipulation of forest ecosystem catchments - the CLIMEX project. IUFRO 18th international meeting for specialists in air pollution effects on forest ecosystems. Abstract book. Institute of Terrestrial Ecology, Edinburgh, U.K, 21-23 September.

Rasmussen, S.K. Antisense suppression and overproduction of peroxidase in barley. COST 824 Conference: Embryogenic potential and transformation of gametic and somatic cells, Newbridge Research Centre, Dublin, Ireland, 23-25 April.

Rasmussen, S.K. The plant as a chemical factory. Risø´s 40th Anniversary, Roskilde, Denmark, 3 June.

Rasmussen, S.K. Transient expression studies of two defense-related barley peroxidases. COST 824, workshop on Advances in Crop Transformation and the Analysis of Transgenic Populations, Norwich, U.K., 1-3 October.

Rasmussen, S.K., Avato, P. Molecular cytogenetic analysis of *Thapsia garganica* genome. 46th Annual Congress of GA, the Society for Medicinal Plant Research: Quality of medicinal plants and herbal medicinal products, Vienna, Austria. 31 August-4 September.

Rasmussen, S.K. Udvikling af transgene byg- og hvedesorter med forøget phytaseaktivitet eller reduceret phytinindhold. Cerealienetværkets Årsmøde, Slagelse, Denmark. 5-6 November. (In Danish).

Ravnskov, S., Nybroe, O., Jakobsen, I. Interactions between *Glomus intraradices* and the plant growth

promoting bacteria *Pseudomonas fluorescens* DF57. Second International Conference on Mycorrhiza, ICOM, Uppsala, Sweden, 5-10 July.

Roberts, T.H., Hejgaard, J., Rasmussen, S.K. Serpin genes are differentially expressed in barley. FEBS Meeting, Copenhagen, Denmark, 5-10 July.

Schmidt, A.S, Pretreatment of lignocellulose for production of convertible carbohydrates. NordPhys Seminar, Oslo, Norway, 23-24 April.

Schmidt, A.S., Thomsen, A.B., Toftegaard, H., Lilholt, H. Polypropylene composites reinforced with wetoxidised wheat straw. 10th European Conference and Technology Exhibition, Würzburg, Germany, 8-11 June.

Schweiger, P., Jakobsen, I. Hyphal phosphorus uptake by native AM fungal populations. Second International Conference on Mycorrhiza, ICOM, Uppsala, Sweden, 5-10 July. (Poster).

Schweiger, P., Jakobsen, I. In vivo AM systems - hyphal P uptake. Workshop on Arbuscular Mycorrhizas in Sustainable Soil-Plant Systems, COST-Action 821, Uppsala, Sweden, 3-4 July.

Stürup, S. Measurement of lead isotopic ratios – A comparison of HR-ICPMS and Q-ICPMS. 1998 Winter Conference on Plasma Spectrometry. 5-10 January. Arizona, USA.

Stürup, S., Kristensen, L.V., Larsen, E. Application of HR-ICPMS for the measurement of trace elements in mussels. 10th Nordic Conference on Mass Spectrometry. Umeå, Sweden. 22-25 August.

Stürup, S., Larsen, E. Determination of trace elements in mussels by Q-ICPMS and HR-ICPMS. 1998 Winter Conference on Plasma Spectrometry, Arizona, USA, 5-10 January.

Saalbach, G., May, M., Dahse, I., Lein, W. Cloning and regulation of plant K^+ channels. Seminar at the Max-Planck-Gesellschaft, Arbeitsgruppe Molekulare and zelluläre Biophysik, Friedrich-Schiller-Universität Jena, Germany, February.

Saalbach, G., Rohrbeck, M., Natura, G., Buschmann, P., Lein, W., Schwerdel, M., Dahse, I., Nagy, F. Regulation of potassium channels by G proteins and 14-3-3 proteins. Seminar at the Lehrstuhl für Genetik, University of Bielefeld, Germany, 11 May.

Saalbach, G., Rosendahl, L., Giese, H. Characterisation of nodulins specifically involved in the function of the symbiosome. First International *Lotus japonicus* Workshop, University of Aarhus, Århus, Denmark, 20-22 November.

Thomsen, A.B., Schmidt, A.S. Recent development on pre-treatment in Denmark - Fractionation of wheat straw into convertible polysaccharides by wet oxidation: Effect of harvest year. Ethanol from Lignocellulose. Young Scientists Conference, Borregaard Manor, Sarpsborg, Norway, 9-11 November.

Thomsen, A.B., Schmidt, A.S. Recent development on pretreatment in Denmark - Wet oxidation in laboratory and pilot scale. Young Scientist Conference, Borregaard Manor, Sarpsborg, Norway, 9–11 November.

Tranekjer, M., Sommer, P., Ahring, B.K., Thomsen, A.B., Schmidt, A.S. Production of bioethanol: Structural characterization of pretreated lignocellulose. Young Scientist Conference, Borregaard Manor, Sarpsorg, Norway, 9–11 November.

Wallington, T.J., Guschin, A., Crawford, M.A., Szente, J.J., Maricq, M.M., Sehested, J., Christensen, L.K., Møgelberg, T., Nielsen, O.J., Orlando, J.J., Tyndall, G.S., Francisco, J.S. The Atmospheric Chemistry of acetyl peroxy radicals: Kinetics and mechanisms of their reactions with NO, NO_2 and HO_2 radicals. 15th International Symposium on Gas Kinetics, Bilbao, Spain, 6-10 September.

Wallington, T.J., Kaiser, E.W., Sehested, J., Møgelberg, T., Nielsen, O.J. Dimethyl ether oxidation: A study of the $CH_3OCH_2 + O_2$ reaction. Third German-Italian Workshop on Tropospheric Chemistry. Bergamo, Italy, 30-31 October.

Worsøe-Møller, C., Egsgaard, H., Grøn, C., Svensmark, B. HPLC og flash pyrolyse GC/MS analyse af lineære alkylbenzenesulfonater (LAS) isomerer. 5. Danske symposium i analytisk kemi (DANSAK 5). Danmarks Farmaceutiske Højskole, Copenhagen, Denmark, 19-20 August. (In Danish).

Worsøe-Møller, C. Flash pyrolyse GC/MS af liniær alkylbenzensulfonater (LAS). Levnedsmiddelkontrollen, Glostrup, Denmark. 9 September. (In Danish).

Worsøe-Møller, C. Flash pyrolyse GC/MS af liniær alkylbenzensulfonater (LAS). Dansk selskab for massespektrometri's årsmøde. University of Odense, 15 October. (In Danish).

Østergård, H. Gene for gene coevolution of cultivated barley and the barley powdery mildew fungus (*Erysiphe graminis* f.sp.*hordei*) ESF Workshop on adaptation of plants to pathogens and herbivores, Kindrogan Field Station, 16-20 September. (Abstract available).

Østergård, H. Use of multilocus selection models in barley powdery mildew for evaluating strategies of disease management. 7th International Congress of Plant Pathology, Edinburgh, Scotland, 9-16 August.

Østergård, H., Hovmøller, M. Virulence survey data - sampling and analysis. NJF Seminar 302, Tune Denmark. 25-27 November. Nordisk Jordbrugsforskning.

11 Papers Accepted for Publication

Ahring, B.K., Licht, D., Schmidt, A.S., Sommer, P., Thomsen, A.B. Production of ethanol frow wet oxidises wheat straw by *Thermoanaerobacter mathranii*, Biores. Technol.: 3-9.

Backes, G., Kicherer, S., Baum, M., Jensen, J., Jahoor, A. Analysis of quantitative resistance loci in barley. NJF-Seminar No. 302, Tune, Denmark, 25-27 November. Nordisk Jordbrugsforskning.

Bibak, A., Behrens, A., Stürup, S., Knudsen, L., Gundersen, V. Concentrations of 63 major and trace elements in Danish agricultural crops measured by inductively coupled plasma mass acectrometry. 1. Onion (*Allium cepa* Hysom). Journal of Agricultural and Food Chemistry.

Bibak, A., Behrens, A., Stürup, S., Knudsen, L., Gundersen, V. Concentrations of 55 major and trace elements in Danish agricultural crops measured by inductively coupled plasma mass spectrometry. 2. Pea (*Pisum sativum* Ping Pong). J. Agricul. Food Chem.

Bibak, A., Stürup, S., Gundersen, P., Gundersen, V. Effect of N-fertilizer levels and soil pH on uptake of trace elements by plants. genetics and molecular biology of plant nutrition, Helsingør. 17-21 August.

Burleigh, S., Harrison, M.J. A gene from *M. truncatula* which is down-regulated by both phosphate fertilization and arbuscular mycorrhizal colonization. In: Phosphores in Plant Biology, Pennstate, University Park, PA (US), 28-30 May.

Burleigh, S.H., Harrison, M.J. The down-regulation of Mt4-like genes by phosphate fertilization occurs systemically and involves phosphate translocation to the shoots. Plant Physiol.

de la Calle-Guntiñas, M.B., Laturnus, F., Adams, F.C. Construction and optimization of a purpe and trap/thermal desorption device for the determination of dimethylselenide and dimethyldiselenide. Fresenius Journal of Analytical Chemistry.

Eriksen, L. Modelling genes for aggressiveness in the *Mycosphaerella graminicolaweat* pathosystem. Resistance biology of agricultural crops. NJF-seminar nr. 302 Tune, Danmark, 25-27 November. Nordisk Jordbrugsforskning.

Feilberg, A., Kamens, R.M., Strommen, M.R., Nielsen, T. Modelling the formation, decay, and partitioning of semivolatile nitro-PAH, nitronaphthalenes, in the atmosphere. Atmos. Environ.

Green, H., Larsen, J., Olsson, P.A., Jensen, D.F., Jakobsen, I. Suppression of the biocontrol agent *Trichoderma harzianum* by external mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* in root-free soil. Applied and Environmental Microbiology.

Grøn, C. Overvågning for miljøfremmede stoffer i vandmiljøet. Vand & Jord.

Hatzack, F., Johansen, K.S., Rasmussen, S.K. Low phytic acid mutants and high phytase crops: Two strategies to improve the availability of phosphate. In: Proceedings of the Sixth International Symposium on Genetics and Molecular Biology of Plant Nutrition, Elsinore, Denmark, 17-21 August.

Hatzack, F., Johansen, K.S., Rasmussen, S.K. Identification and characterisation of barley grain mutants with a low content of phytic acid. In: Proceedings of the Workshop on the Biochemistry of Plant Phytate and Phytases, Copenhagen, Denmark, 25-28 October 1997.

Jahoor, A., Park, R.F., Burdon, J.J. Evidence for somatic hybridisation in the leaf rust pathogen (*piccinia recordita* f.sp. *Tritici*) of wheat (*Tritricum aestivum*) in nature. Mycol. Res.

Jakobsen, I., Smith, A.F., Schweiger, P.F., Smith, S.E. The influence of arbuscular mycorrhizal fungi on phosphate uptake by roots. In: Noble Foundation Plant Biology, 10 year Symposium.

Jensen, L.G., Pulitz, O., Olsen, O., Thomsen, K.K., von Wettstein, D. Inheritance of a codon-optimized transgene expressing heat stable (1,3-1,4)—glucanase in scutellum and aleurone of germinating barley. Hereditas 129.

Kollist, H., Moldau, H., Rasmussen, S.K., Mortensen, L. The effect of reduced light and ozonevon apoplastic ascorbate and photosynthesis in the leaves of barley. Proc. XI congress of Photosynthesis, Budapest, Hungary. Kluwer Acad.

Kristensen, B.K., Block, H., Rasmussen, S.K. Barley coleoptile peroxidases: purification, molecular cloning and induction by pathogens. Plant Physiol.

Linde-Laursen, I., Bothmer, R. von Orderly arrangement of the chromosomes within barley genomes of chromosome-eliminating *Hordeum lechleri* x barley hybrids. Genome.

Mehrtens, G., Laturnus, F. Mixed function oxidase dependent biotransformation of polychlorinated biphenyls by different species of fish from the North Sea. Chemosphere.

Ravnskov, S., Jakobsen, I. Effects of *Pseudomonas fluorescens* DF 57 on growth and P uptake of two arbuscular mycorrhizal fungi in symbiosis with cucumber. Mycorrhiza.

Ravnskov, S., Larsen, J., Olsson, P.A., Jakobsen, I. Effects on various organic compounds on growth and phosphorus uptake of an arbuscular mycorrhizal fungus. New Phytologist.

Ravnskov, S., Nybroe, O., Jakobsen, I. Influence of an arbuscular mycorrhizal fungus on *Pseudomonas fluorescens* DF 57 in rhizosphere and hyposphere soil. New Phytologist.

Schwarz, G., Herz, M., Huang, X., Jahoor, A., Wenzel, Cr., Mohler, V. Application of fluoresancebased semi-automotic RFLP-analysis in barley and wheat. Biotechnol.

Schwarz, G., Michalek, W., Mohler, V., Wenzel, C., Jahoor, A. Chromosome landing at the mla locuss in barley (*Hordeum vulgare* L.) by means of high resolution mapping with RFLP markers. Theor. Appl. Crenet.

Schweiger, P.F., Jakobsen, I. The role of mycorrhizas in plant P nutri-

tion: Fungal uptake kinetics and genotype variation. In: Proc. of the 6th International Symposium on Genetics and Molecular Biology of Plant Nutrition, Kluwer.

Schweiger, P.F., Thingstrup, I., Jakobsen, I. Comparison of two test systems for measuring plant phosphorus uptake via arbuscular mycorrhizal fungi. Mycorrhiza.

Sehested, J., Christensen, L.K., Nielsen, O.J., Wallington, T.J. Absolute rate constants for $F+CH_3CHO$ and CH_3CO+O_2, relative measurement of CH_3CO+NO and the product distribution of the $F+CH_3CHO$ reaction. Int. J. Chem. Kinet.

Simonsen, A.C.W., Rosendahl, L. Origin of de novo synthesized proteins in the different compartments of pea-*Rhizobium* symbiosomes. Molecular Plant-Microbe Interaction.

Slaets, S., Adams, F.C., Laturnus, F. GC coupled to MIP AES applied to the speciation analysis of volatile halocarbons. LC·GC International.

Slaets, S., Laturnus, F., Adams, F.C. Microwave induced plasma atomic emission spectrometry - A suitable detection system for the determination of volatile halocarbons. fresenius. Journal of Analytical Chemistry.

Snow, A.A., Andersen, B., Jørgensen, R.B. Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *Brassica rapa*. Molecular Ecology.

Sokolov, O., Hurley, M.D., Wallington, T.J., Kaiser, E.W., Platz, J., Nielsen, O.J., Berho, F., Rayez, M.-T., Lesclaux, R. Kinetics and mechanism of the gas phase reaction of Cl atoms with benzene. J. Phys. Chem.

Saalbach, G., Natura, G., Lein, W., Buschmann, P., Dahse, I., Rohrbeck, M., Nagy, F. The a-subunit of a heterotrimeric G-protein from tobacco, NtGPa1, functions in potassium channel regulation in mesophyll cells. J. Exp. Bot.

Wallington, T.J., Nielsen, O.J. Atmospheric degradation of anthropogenic molecules. In: The Handbook of Environmental Chemistry Vol. 2 (ed. P. Boule), Springer-Verlag.

Weidler, P.G., Hug, S.J., Wetche, T.P., Hiemstra, T. Determination of growth rates of (100) and (110) faces synthetic goethite by scanning force microscopy. Geochimica et Cosmochimica Acta.

Østergård, H., Hovmøller, M. Virulence survey data - sampling and analysis. Seminar 302, Tune, Danmark 25-27 November, Nordisk Jordbrugsforskning.

12 Education

12.1 Ph.D. Theses

Ravnskov, S. (1998) Interactions between Arbuscular Mycorrhizal Fungi and Saprotrophic Microorganisms in Soil. Ph.D. Thesis. Risø National Laboratory and The Royal Veterinary and Agricultural University, Copenhagen, Denmark. 79 p.

Simonsen, A.C.W. (1998) Origin of de novo Synthesized Proteins in the Interface between Pea-*Rhizobium* in Root Nodules. Ph.D. Thesis. Risø National Laboratory and The Royal Veterinary and Agricultural University, Copenhagen, Denmark. 87 p.

Butterworth, L. (1998) Interactions of the pathogen *Leptosphaeria maculans* (Desm.) Ces & de Not. and *Brassica napus*. Ph.D. Thesis. Risø National Laboratory and Department of Brassicas and Oilseed Research, John Innes Centre, Norwich, U.K.

Christensen, L.K. (1998) Atmospheric Chemistry of Volatile Organic Compounds. Ph.D. Thesis. Risø National Laboratory and University of Copenhagen.

Fauser, P. (1998) Particulate Air Pollution, with Emphasis on Traffic Generated Aerosols. Ph.D. Thesis. Risø National Laboratory and Technical University of Denmark.

Kristensen, B.K. (1998) Physiological and Molecular Characterisation of Prx7 and Prx8, Two Defence-Related Peroxidases from Barley. Ph.D. Thesis. Risø National Laboratory and The Royal Veterinary and Agricultural University, Denmark. 104 p.

12.2 M.Sc. Theses

Monrad, A. (1998) Identification and Characterization of *Erysiphe graminis* f.sp. *hordei* Genes that are Expressed during the Symbiosis with Barley, Risø National Laboratory and University of Odense.

Johannessen, M. (1998) Genetic Variation and CO_2 Exploitation in Winter Varieties of Oilseed Rape *(Brassica napus)*. M.Sc. Thesis, Roskilde University and Risø National Laboratory. 199 p.

Eriksen, R.Ø. (1998) Transformation i byg for opnåelse af øget lysin- og threoninindhold. M.Sc. Thesis, Risø National Laboratory and The Royal Veterinary and Agricultural University, Copenhagen, Denmark. 90 p.

Rudbeck, A. (1998) Transport af aspartat over symbiosommembranen isoleret fra ærterodknolde. Risø National Laboratory and University of Roskilde, Denmark. 99 p.

Eydesgaard, E.J, Larsen, V.H. (1998) Termisk og katalytisk omdannelse af tjæreforbindelser dannet ved pyrolyse af biomasse. Hovedrapport og appendiks- og bilagsrapport. Risø National Laboratory and Technical University of Denmark.

12.3 External Examiners

Gissel-Nielsen, G. Censor in plant nutrition and crop physiology at The Royal Veterinary- and Agricultural University, Copenhagen.

Grøn, C. Member of the panel of external examines at the Danish Engineering Educations.

Gundersen, V. Censor in chemical analysis at Technical University of Denmark.
Censor in mechanical engineering at Aalborg University.

Jensen, A. Censor in biology at all Danish universities.

Jakobsen, I. Censor at University of Copenhagen.

Jørgensen, R.B. Censor in molecular biology at University of Aarhus.

Nielsen, O.J. Censor in chemistry at University of Copenhagen and University of Odense.

Pilegaard, K. Censor in ecology, University of Copenhagen.

Pilegaard, K. Censor in air pollution, Technical University of Denmark.

Rasmussen, S. Censor at The Royal Veterinary and Agricultural University, Copenhagen.

Rosendahl, L. Censor in plant nutrition at The Royal Veterinary Copenhagen.

Østergård, H. Censor in biology at University of Aarhus and University of Copenhagen.

12.4 External Teaching and Lectures

Christiansen, S.K. Lectures in molecular plant path at The Royal Veterinary and Agricultural University, Copenhagen 7 February.

Eriksen, L. Lecture at The Royal Veterinary and Agricultural University, Copenhagen 24 February.

Jahoor, A. Lecture "Molecular markers for resistance breeding in cereals" at The Royal Veterinary and Agricultural University, Copenhagen 3 December.

Jakobsen, I. Lectures at The Royal Veterinary and Agricultural University, Copenhagen 25 February and 28 April.

Rosendahl, L. Lectures in Advances in Plant Nutrition at The Royal Veterinary and Agricultural University, Copenhagen 28 April.

Rosendahl, L. Lectures in Plant Biochemistry Nutrition at The Royal Veterinary and Agricultural University, Copenhagen 10 November.

Pedersen, C. Lectures in molecular plant path at The Royal Veterinary and Agricultural University, Copenhagen 7 February.

Rasmussen, S.K. Lecture in Plant Biochemistry at The Royal Veterinary and Agricultural University, Copenhagen 24 November.

Stürup, S. Course of Chemistry 3AØ at the University of Copenhagen.

Østergård, H. Lecture "Modeller i epidemiologi og populationsgenetik" at The Royal Veterinary and Agricultural University, Copenhagen 12 November.

13 Exchange of Scientists

Abramova, K.B. Ioffe Physical Tech. Institute, St. Petersburg, Russia (4 months).

Backes, G. stationed at Technische Universität München, Freising-Weihenstephan, Germany (3 months).

Bascones, E. Ciudad Universitaria, Madrid Spain (3 months).

Bobrowski, K. Institute of Nuclear Chemistry and Technology, Warsaw, Poland (5 weeks).

Bousset, L. INRA, Grignon, France (3 weeks).

Burbridge, E. University of Dublin, Ireland (4 weeks).

Christensen, L.K. leave of absence as Post doc at Ford Motor Company, Dearborn, USA (4 months).

Germon, F. Ecole Superieure Dágriculture, France (3 months).

Germon, F. Ecole Superieure Dágriculture, France (5 months).

Glasby, G.P. University of Sheffield, U.K. (1 month).

Jahoor, A. Leave of absence at Technische Universität München, Freising-Weihenstephan, Germany (3 months).

Jacobsen, F. HOH Water Technology, Denmark (9 months).

Kovacs, A. Radiation Chemistry Department, Budapest, Hungary (2 weeks).

Lyngkjær, M. Leave of absence as Post doc at Institute of Grassland and Environmental Research (IGER), Aberystwyth, U.K. (12 months).

McLaughlin, W.L. National Institute of Standards and Technology, USA (1 week).

Mouritzen, P. Technical University of Denmark (12 months).

Olsson, P.A. University of Lund, Sweden (4 months).

Ovesna, J. Research Institute for Crop Production, Ruzyne, Praha, Czechoslovakia (1 month).

Purnhauser, L. Cereal Research Institute, Szeged, Hungary (1 month).

Roberts, T.H. Technical University of Denmark (6 months).

Schmidt, A.S. visiting scientist at VTT Biotechnology and Food Research, Espoo, Finland (3 weeks).

Shim, Sang In Department of Agronomy, College of Natural Resources, Korea University, Seoul, Korea (6 months).

Smith, A. Department of Botany, The University of Adelaide, Australia (5 months).

Smith, S.E. Department of Soil Science, The University of Adelaide, Australia (5 months).

Tomiuk, J. Klinische Genetik Universität Tübingen, Germany (6 months).

Wu, Boqian, National Center fpr Gene Research, Chinese Academy of Sciences, Shanghai, P.R. China (12 months).

14 Guest Lectures

Rosendahl, S. University of Copenhagen, Denmark: "Populationsgenetiske studier af arbuskulære mykorrhizasvampe" (in Danish), 5 March.

Kahns, L. University of Aarhus: "Surface display of melanoma cDNA products on filamentous phage using the pJuFo system", 12 March.

Mølhøj, M. Danish Technical University: "En membranbundet endo-1,4-—glucanase (Cel 16) fra raps tilhørende en ny undergruppe", 19 March.

Finckh, M. The Royal Veterinary and Agricultural University, Copenhagen, Denmark: "Biodiversity as a crucial factor in ecological plant protection", 2 April.

Lohse, C. University of Odense, Denmark: "^{14}C metode til bestemmelse af biogene/ anthropogene emissioner" (in Danish), 16 April.

Guldbrandtsen, B. Danish Institute of Agricultural Sciences, Flakkebjerg, Denmark: "Loss of genetic variation with marker assisted selection in outbreeding species", 23 April.

Ingerslev, M. Danish Research Institute for Forest and Landscape: "Vitalitetsgødskning af vestjydske granplantager - gødskning for vækst og sundhed" (in Danish), 30 April.

*Ortiz, R.*The Royal Veterinary and Agricultural University, Copenhagen, Denmark: "An evolutionary breeding scheme for triploid plantains and bananas ensuing from genetic analysis", 14 May.

Ramos, L.P. Federal University of Paraná, Curitiba, Brazil: "Pretreatment and hydrolysis of biomass", 18 May.

Flyvbjerg, H. Niels Bohr Institute, Denmark: "Dynamics of Microtubules", 3 September.

Binnerup, S. National Environmental Research Institute, Denmark**:** **"**Micro-biological agents for control of plant diseases. Possibilities, limitations and potential risks", 17 September.

Karlson, U. National Environmental Research Institute, Denmark: "Bioremediation of PAH", 8 October.

Smith, S., Smith, A. University of Adelaide, Australia: "Mycorrhizal interfaces – structure and function", 29 October.

Bjørn, S. Novo Nordisk, Denmark: "GFP studies. Making the inviable viable", 5 November.

Bjerregård, P. University of Odense, Denmark: "Østrogen lignende kemikalier" (in Danish), 3 December.

Fischbeck, G. Technical University of Munich. Germany: "Use of genetic resources in cereal breeding", 3 December.

Helweg, A. Danish Institute of Agricultural Sciences, Flakkebjerg, Denmark: "Hvad ved vi om pesticiders skæbne i luften?" (in Danish) 10 December.

15 Committee Membership

15.1 National

Giese, H. OECD Committee for Biotechnology.
Board of Danish Polar Center.
Danish Society for the Conservation of Nature, Committee for Environmental Issues.

Gissel-Nielsen, G. Danish Academy of Technical Sciences.
Research Center for Organic Farming.
Danish Society for the Conservation of Nature, Committee for Environmental Issues.

Grøn, C. Board of the Soil and Ground Water Contamination.
Commitee of the Danish Council of Technical Sciences.
Member of the steering committee of the Bioventilation Centre (CEVENT) under the Danish Ministry of Industry (a DCR centre).
Member of the interinstitutional contact group of the Danish R&D Centre for Decontamination of Soil and Sediment (DCR).
Member of the editorial committee for Journal of Environmental Science and Health, Part A, and for the Danish journal Vand & Jord (Water and Soil).
Head of Centre for Sustainable Land Use and Management of Contaminants, Carbon and Nitrogen, an open centre under the Danish Environmental Research Programme, 1997-2000.

Jahoor, A. Member of Danish Gene Bank Committee.

Jensen, A. Member of the board of directors for Pajbjerg Foundation, Denmark.
DCAR, Danish Center of Atmospheric Research.
DaFoLa, Danish Center for Forest and Landscape Ecosystem Research.
Chairman of the evaluation panel conserning aquatic and terrestrial ecotoxicology in The Danish Environmental Research Programme.
Member of The Danish Academy of Technical Sciences.

Jørgensen, R.B. Member of the Danish Environmental Appeal Board.
Board of Centre for Effects and Risks of Biotechnology in Agriculture (The Danish Environmental Research Programme.

Larsen, E. Danish Society of Mass Spectrometry.
The Danish National Committee for Chemistry.

Miller, A. Danish Medical Device Association (DMDA).
Sterilization committee.
Danish Standards Association.
Committee S 259 on Sterilization of Medical Equipment. (Chairman).

Nielsen, O.J. Member of the Danish Natural Science Research Council.
Nielsen, T. Board member of the Centre of Biological Processes in Contaminated Soil and Sediment.

Rasmussen, S.K. Society of Danish Engineers, Board of Chemistry Section.
Board of Danish Cereal Association.
Board of Chemistry Section, Society of Danish Engineers.

Østergård, H. Ph.D. Committee for Education, The Royal Veterinary and Agricultural University, Copenhagen.
Chairperson for COST 817 Management Committee on Population Studies of Airborne Pathogen on Cereals as a Mean of Improving Strategies for Disease Control.
Coordination group for resistance and virulence in cereals and cereal pathogens in Denmark.
Board of Centre for Effects and Risks of Biotechnology in Agriculture (The Danish Environmental Research Programme.

15.2 International

Ambus, P. U.S. Trace Gas Network (TRAGNET), working group.

Giese, H. Editorial board, Hereditas and Plant Pathology on line.
OECD committee for Biotechnology.

Grøn, C. Board member of the Nordic branch of the International Humic Substances Society.

Jakobsen, I. Member of Advisory Board for New Phytologist.

Jørgensen, R.B. Member of Panel of Experts, International Biosafety Forum, Third World Network.
Larsen, E. European Commission, Access to Large-Scale Facilities, Technical Audit of the HCM-LSF contracts.

Miller, A. Editor-in-Chief Radiation Physics and Chemistry.
International Electrotechnical Commission (IEC), Subcommittee 15B, Working Group 2 on Endurance Tests. Radiation. Corresponding member.
Organisation Internationale de Metrologie Legale (OIML). TC-15. Measuring measurements for ionizing radiation. Member.

Pilegaard, K. Member of steering committee for BIATEX-2 under EUROTRAC-2.

Rasmussen, L. Member of the Swedish Research Council for Forestry and Agriculture, Section for Biogeochemistry.
Member of Editorial Board for Forest Ecology and Management.

Thomsen, A.B. IEA - Bioenergy agreement Task XIII. Bioconversion activity. Alternative member of committee.

Østergård, H. COST, chairman of Management Committee "Population studies of airborne pathogens on cereals as a means to improve strategies for disease control".
Editorial board of Agronomie.

16 Seminars and Courses Organized

40-Years of Research at Risø: A Platform for the Future Interacting with Industry and Society. Risø National Laboratory 3 June (*L. Rosendahl, L. Lading*).

Ph.D.-course "Molecular Markers in Plant Genetics and Plant Breeding", Risø National Laboratory 8-19 June (*G. Backes, A. Jahoor*).

Development on a Function of the Mycelium of Arbuscular Mycorrhizal Fungi. Preconference Workshop in Uppsala 1-2 July (*I. Jakobsen, P. Schweiger*).

Methods for Studying the Physiology of Nutrient Transport in Arbuscular Mycorrhizas, COST 821 Workshop, Uppsala 3-4 July (*I. Jakobsen, P. Schweiger*).

Training Course on Validation and Process Control for Electron Beam Sterilization, Risø National Laboratory 15-19 June *(A. Miller)*.

6th International Symposium on Genetics and Molecular Biology of Plant Nutrition, LO-Skolen, Elsinor, Denmark 17-22 August (*G. Gissel-Nielsen, A. Jensen*).

Training Course on Validation and Process Control for Electron Beam Sterilization, Risø National Laboratory 17-21 August *(A. Miller)*.

Chemical Mechanisms of Atmospheric Processes. EC Workshop Copenhagen, Denmark 24-25 August (*O.J. Nielsen*).

6th FECS Conference on Atmospheric Chemistry and Air Pollution, H.C. Ørsted Institute, University of Copenhagen, Denmark 26-28 August (*O.J. Nielsen*).

Ph.D. course "Seminars in Plant Microbe Symbiosis", October-December (*H. Giese, L. Rosendahl, I. Jakobsen, S.K. Christiansen, G. Saalbach*).

Conference on Organic Waste Products in Sustainable Land Use, Ingeniørhuset, Copenhagen 16 November (*C. Grøn*).

17 Scientific Results and Finances

17.1 Scientific Results

Plant Biology and Biogeochemistry Department		
	Results 1997	**Results 1998**
Basic research and development (man-month)	361	297
Research programmes (man-month)	781	877
Commercial contracts (man-month)	77	38
Technical support for research (man-month)	66	19
Management (man-month)	118	69
Total	1403	1381
Dissemination of results		
Papers in international journals and books	84	97
Papers in Danish journals and books	17	11
Risø reports, R , M og I	6	7
Danish books and reports	11	7
International books and reports	4	5
Other publications	6	8
Papers in conference proceedings	30	16
Other internationale conference contributions	90	95
Other Danish conference contributions	30	44
Patent proposals	0	1
Networking and collaboration		
Ph.D. students (number)	21	29
Ph.D. degrees (number)	5	6
Risø leave of abcence (man-month)	21	25
Visiting scientists at Risø (man-month)	29	37
Scientific papers reviewed (number)	162	93
Committees for Ph.D.thesis, promotion of scientists, senior scientists, professors (number)	17	28
Committee memberships (number)	36	31
Collaboration with companies (man-month)	172	258
Contribution from companies in projects (man-month)		166
Collaboration with research institutes (man-month)	462	368
Contribution from institutes in collaboration (man-month)		662
Collaboration with public authorities (man-month)	7	3

17.2 Finances

The activities of the Department are supported by a combination of government appropriations, project funds from national and international research programmes and fully commercial industrial contracts.

1998	DKK 1000	USD 1000
Income		
Dept.'s share of Risø's government appropriations	37,326	5,497
Programmes and contracts	37,878	5,578
Durable equipment	1,720	253
Total	76,924	11,329
Expenditure		
Salaries	54,317	8,000
Operating expenses	14,626	2,154
Durable equipment	7,981	1,175
Total	76,924	11,329

Additional funding has been obtained for Ph.D. grants, Post. doc. fellowships, apprentices and trainees.

18 Personnel

The research activities are organized into 6 research programmes and supported by special facility units.

The Department includes 71 full time scientific staff members and 46 full time technical staff members.
The list also includes short-term employees.

Head of Department:
Arne Jensen

Research Programmes
Plant-microbe Symbiosis
Head: Henriette Giese.
Plant Products and Products Quality
Head: Søren Rasmussen (constituted).
DLF-Risø Biotechnology
Head: Klaus K. Nielsen.
Plant Genetics and Epidemiology (Including the experimental farm, Dyskærgård and field experiments)
Head: Hanne Østergård.
Biogeochemistry
Head: Lennart Rasmussen (constituted).
Plant Ecosystems and Nutrient Cycling (Including growth chambers, Risø Environmental Risk Assessment Facility (RERAF), green-houses).
Head: Gunnar Gissel Nielsen.

Special Facility
Risø High Dose Reference Laboratory
Head: Arne Miller.
Risø Integrated Environmental Facility (RIMI)
Head: Kim Pilegaard.

18.1 Scientific staff

Ambus, Per
Andersen, Claus H.
Backes, Gunter
Baunsgaard, Lone
Beier, Claus
Bibak, Allan (until 31.08)
Bjergbakke, Erling
Burleigh, Steven
Christensen, Lene Krogh
Christiansen, Solveig Krogh
Didion, Thomas
Egsgaard, Helge
Engvild, Kjeld C.
Fenger, Jørgen
Gavito, Mayra
Giese, Henriette
Gissel Nielsen, Gunnar
Grøn, Christian
Gundersen, Pernille (until 31.03)
Gundersen, Vagn
Habibzadeh, Alireza (until 10.07)
Hatzack, Frank
Herly, Lene (until 31.08)
Holcman, Jerzy
Haahr, Vagner (until 31.03)
Jahoor, Ahmed
Jakobsen, Iver
Jensen, Erik Steen
Jensen, Jens
Jensen, Lisbeth Gath
Jørgensen, Bjarne (until 14.08)
Jørgensen, Finn (until 28.02)
Jørgensen, Rasmus (until 31.08)
Jørgensen, Rikke Bagger
Kilen, Hans Henrik (until 18.09)
Kunzendorf, Helmar
Kure, Liv
Larsen, Elfinn
Laturnus, Frank
Lynggård, Bent
Lyngkjær, Michael
Mikkelsen, Teis Nørgaard
Miller, Arne
Mortensen, Gerda Krog
Nielsen, Klaus K.
Nielsen, Ole John
Nielsen, Torben
Nilsson, Karen
O'Hara, Robert (until 31.03)
Pagsberg, Palle
Pedersen, Carsten
Pilegaard, Kim
Rasmussen, Lennart
Rasmussen, Søren Kjærsgård
Ravnskov, Sabine
Richter, Hannes
Rosendahl, Lis
Saalbach, Gerhard
Schmidt, Anette Skammelsen
Schweiger, Peter
Sehested, Jens (until 28.02)
Sehested, Knud
Sillesen, Alfred (until 31.03)
Stürup, Stefan
Thomsen, Anne Belinda
Woidemann, Anders
Østergård, Hanne

18.2 Technical Staff

Andersen, Bente
Andersen, Margit Elm
Brandt, Lis
Brink-Jensen, Merete
Christensen, Gertrud
Corfitzen, Hanne
Djurdjevic, Stanko
Fernqvist, Tomas
Fosskov Jensen, Jette
Gade, Poul (until 30.06)
Gudiksen, Peter
Hansen, Ina
Hansen, Ivan
Hasselbalch, Finn
Ibsen, Elly
Jensen, Birgit
Jensen, Ellen Møller
Jensen, Linette Munksgaard
Karlsen, Aage (until 14.08)
Koutras, Charlotte
Kvamm, Birte (until 31.03)
Larsen, Erik Engholm (until 31.08)
Larsen, Ingelis
Larsen, Inge Merete
Larsen, Tina Bøgeskov
Meltofte, Liselotte
Møller, Anette
Møller, Trine
Nielsen, Anja Christina
Nielsen, Jette Bruun
Nielsen, Vagn Aage
Olsen, Anette
Olsen, Anne
Olsen, Inge
Petersen, René
Sillesen, Anerikke
Storm Petersen, Anne-Mette
Sørensen, Poul
Tung, Tran Duc Tuan
Thygesen, Maria (until 30.06)
Vestesen, Hans
Vinther Kristensen, Lis
Wojtaszewski, Hanne

18.3 Administrative Staff

Bay, Kirsten
Borring Sørensen, Marit
Bækmark, Anni (until 14.08)
Christiansen, Krista
Frandsen, Anette
Hansen, Karina (until 05.11)
Hjorth, Aase
Jakobsen, Inger (until 31.01)
Jensen, Hanne
Krogh, Helle
Lilholt, Ulla
Petersen, Lis

18.4 Ph.D. Students

Christensen, Lene Krogh
Eriksen, Lars B.
Fauser, Patrik
Feilberg, Anders
Frøsig, Lars
Gavnholt, Britta
Grell, Morten
Hauggaard-Nielsen, Henrik
Holst, Pia Bachmann
Jensen, Christian Sig
Johannessen, Marina
Johansen, Katja Salomon
Johansen, Runa Ulsøe
Jørgensen, Rasmus Nyholm
Klinke, Helene B.
Kristensen, Brian K.
Møller, Marianne Gellert
Nielsen, Jock
Petersen, Klaus
Platz, Jesper
Rasmussen, Nanna
Ravnskov, Sabine
Scharff, Anne Marie
Simonsen, Anna Carina Wiborg
Storgaard, Morten
Borgen, Anders

18.5 M.Sc. and B.Sc. Students

Bondo-Larsen, Louise
Borch, Thomas
Broeng, Stine
Christensen, Hasse Dyhr
Dræby, Ingrid
Eriksen, Rasmus
Hansen, Lise
Haselmann, Kim
Jensen, Henrik Østergaard
Johannessen, Marina
Milandt, Jan
Mirza, Almas
Monrad, Annette
Møller, Carsten W.
Nylev, Peter
Pedersen, Lars Meinild
Pertl, Maria
Poulsen, Morten
Rudbeck, Annette
Stein, Thomas N.N.

18.6 Apprentices

Abdellahi, Ebtisan
Andersen, Karina
Arltoft, Kasper Buus
Hansen, Lisbeth
Heinvig, Tania
Jensen, Jimmi Spangenberg
Jespersen, Tina Grith
Kristensen, Henrik
Madsen, Henrik Weinrich
Nielsen, Christian Leon
Olsen, Carina Nykjær
Thorsgaard, Birgitte
Udbjørg, Charlotte

19 Acronyms

AAT	Aspartate AminoTransferase
2-D	Two-Dimensional
AFLP	Amplified Fragment Length Polymorphism
BAC	Bacterial Artificial Chromosome
BASYS	Baltic Sea System Study
CLIMEX	Climate Change Experiment
CLIMOOR	Climate driven changes in the functioning of heath and Moorland ecosystems
CONFIRM	Centre for Continuos Flow Isotope Ratio Mass spectrometry
DANAK	Danish Accreditation Scheme
DDRT-PCR	Differential Display Reverse Transcriptase Polymerase Chain Reaction
DEHP	Di-ethyl-hexyl-phthalate
DH	Chromosome-Doubled Haploid
DIAS	Danish Institute of Agricultural Sciences
DNA	DioxyriboNucleicAcid
DTU	Danish Technical University
EST	Expressed Sequence Tag
EUROFLUX	Effects of CO_2 exchange over European forests
EXAMINE	Exchange of Atmospheric Ammonia with European ecosystems
FA	Fulvic Acid
FOREXNOX	Effectc of nitrogen oxides on European forests
FTIR	Fourier Transform Infra-Red
FØTEK	Danish Food Technology and Development Programme
GC-MS-MS	Gas Chromatography/Mass Spectrometry
GFP	Green Fluorescent Protein
HA	Humic Acid
HDRL	High Dose Reference Laboratory
HFE	HydroFluoroEther
HPLC	High Pressure Liquid Chromatography
HR-ICPMS	High Resolution Inductively Coupled Plasma Mass Spectrometry
IGER	Institute of Grassland and Environmental Research
LAS	Linear Alkyl benzene Sulfonates
MAST	Marine Science and Technology Programme (under CEC)
MDD-HPLC	Metal Dye Detection-High Pressure Liquid Chromatography
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
NPL	National Physical Laboratory
PAC	Polycyclic Aromatic Compounds
PAH	Polycyclic Aromatic Hydrocarbons
PBS	PeriBacteroid Space
PEG	PolyEthylen Glycol
PLS	Discriminant Partial Least Squares regression
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA Technique
REA	Relaxed Eddy-Accumulation
RERAF	Risø Ecological Risk Assessment Facility
RIMI	Risø Integrated Environmental Project
RFLP	Restriction Fragment Length Polymorphism
RNA	RiboNucleicAcid
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
RVAU	The Royal Veterinary and Agricultural University
SDS	Sodium Dodecyl Sulfat
SM	Symbiosome Membrane
SMP	Danish Strategic Environmental Research Programme
SOROFLUX	Effects of land use and organic waste application on carbon and nitrogen fluxes
SSR	Single Sequence Repeat
THOR	Technology by Highly Orientated Research
TLC	Thin Layer Chromatography

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Abstract (max. 2000 characters)

The annual report from the Plant Biology and Biogeochemistry Department aims to provide a summary of our research and achievements and to give an idea of the research directions in the Department.
The Department is engaged in research to establish the scientific basis for new methods in industrial and agricultural production. Through basic and applied experimental research, the Department aspires to develop methods and technology for industrial and agricultural production, exerting less stress and strain on the environment. The research approach in the Department is mainly experimental. In the autumn of 1997 it was decided to reorganize and expand the Department and in 1998 the Department includes six research programmes and special facilities.
Selected departmental research activities during 1998 are introduced and reviewed in seven chapters: 1. Introduction, 2. Plant-Microbe Symbioses, 3. Plant Products and Recycling of Biomass, 4. DLF-Risø Biotechnology, 5. Plant Genetics and Epidemiology, 6. Biogeochemistry, 7. Plant Ecosystems and Nutrient Cycling. The Department's contribution to education and training are presented. Lists of publications, papers accepted for publications, guest lectures, exchange of scientists, lectures and poster presentations at international meetings are included in the report. Names of the scientific and technical staff members, visiting scientists, Postdoctoral fellows, Ph.D. students, M.Sc. students and apprentices are also listed.

Descriptors INIS/EDB

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Risø National Laboratory carries out scientific and technological research in order to create new technological development. The results of Risø's research are used by Danish and international industry, governmental bodies and international organisations. Risø contributes to education of scientists through Ph.D. and postdoctoral programmes.

Risø reports its activities in 1998 in the following publications: Risø Annual Report (available in Danish and English), Annual Performance Report (only available in Danish), Risø's Publication Activities and the annual progress reports of the seven research departments. The publications and further information about Risø can be obtained from the web site www.risoe.dk. Printed copies of the reports are available from the Information Service Department, phone +45 4677 4004, email risoe@risoe.dk, fax +45 4677 4013.